Cleavage sites of endoproteases on the a-chain of GPIb

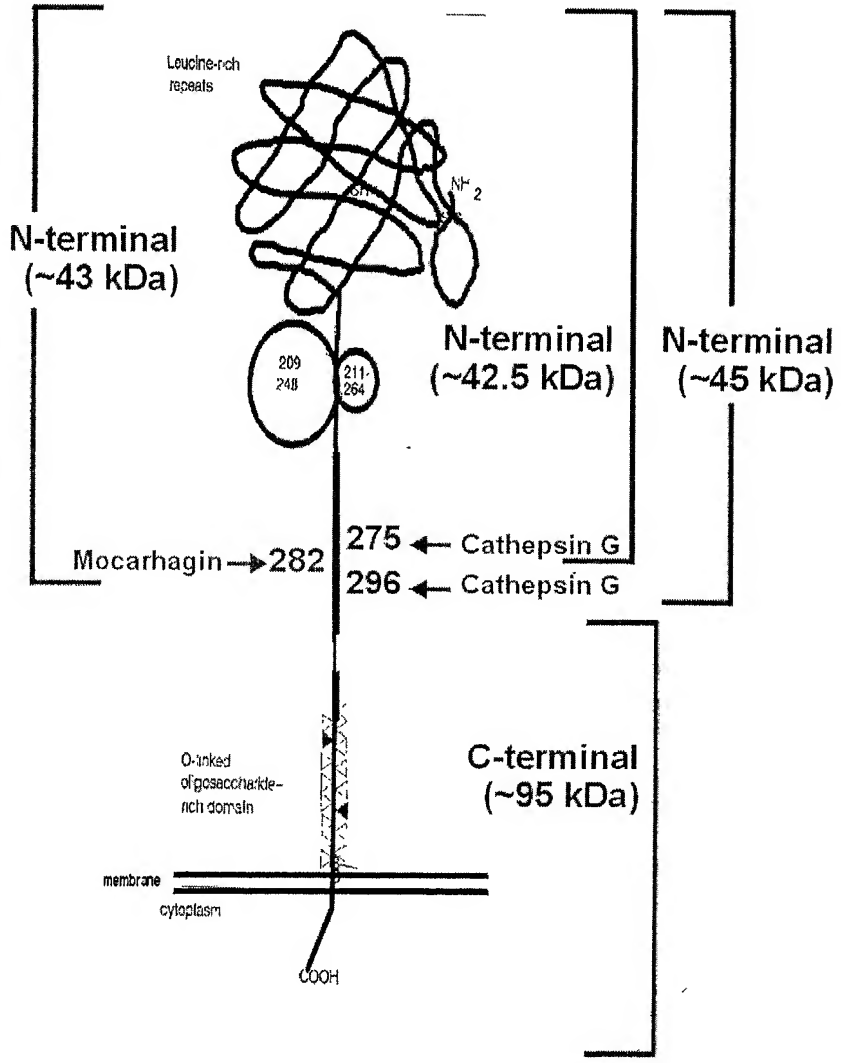


FIG. 1

Binding of Y1 and Y17 to platelets in reduced and non-reduced conditions

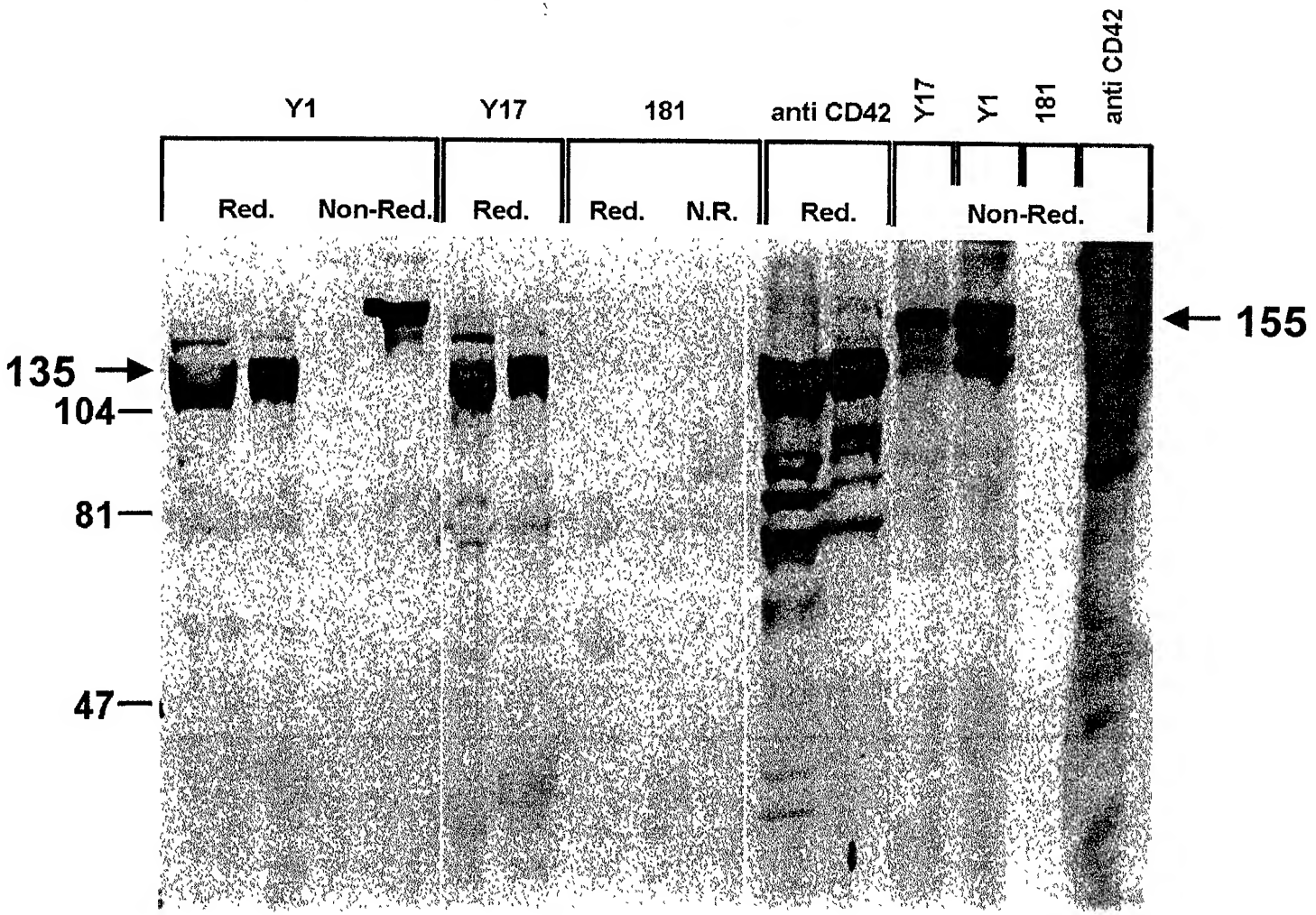


FIG. 2

Characterization of Optimal Determinants for Binding of Y1 to It's Ligands

	Platelets/GC	KG1/RP-HPLC #4
Rec: GP1b 1-340	-	
GP1b 1-480	-	
Glycanase: N	+	+++
N+O	+	+++
Proteases: Mocarhagin	++ (~40kD)	-
O-Sialo Peptidase	++ (~40kD)	-
Ficin	-	-
Trypsine	++ (~40kD)	-
Elastase	++ (~40kD)	++
Sulfatase (Aryl)		-/+

FIG. 3

Cleavage of platelets GPIb by O-Sialoglycoprotein abolishes binding of both Y1 and Y17

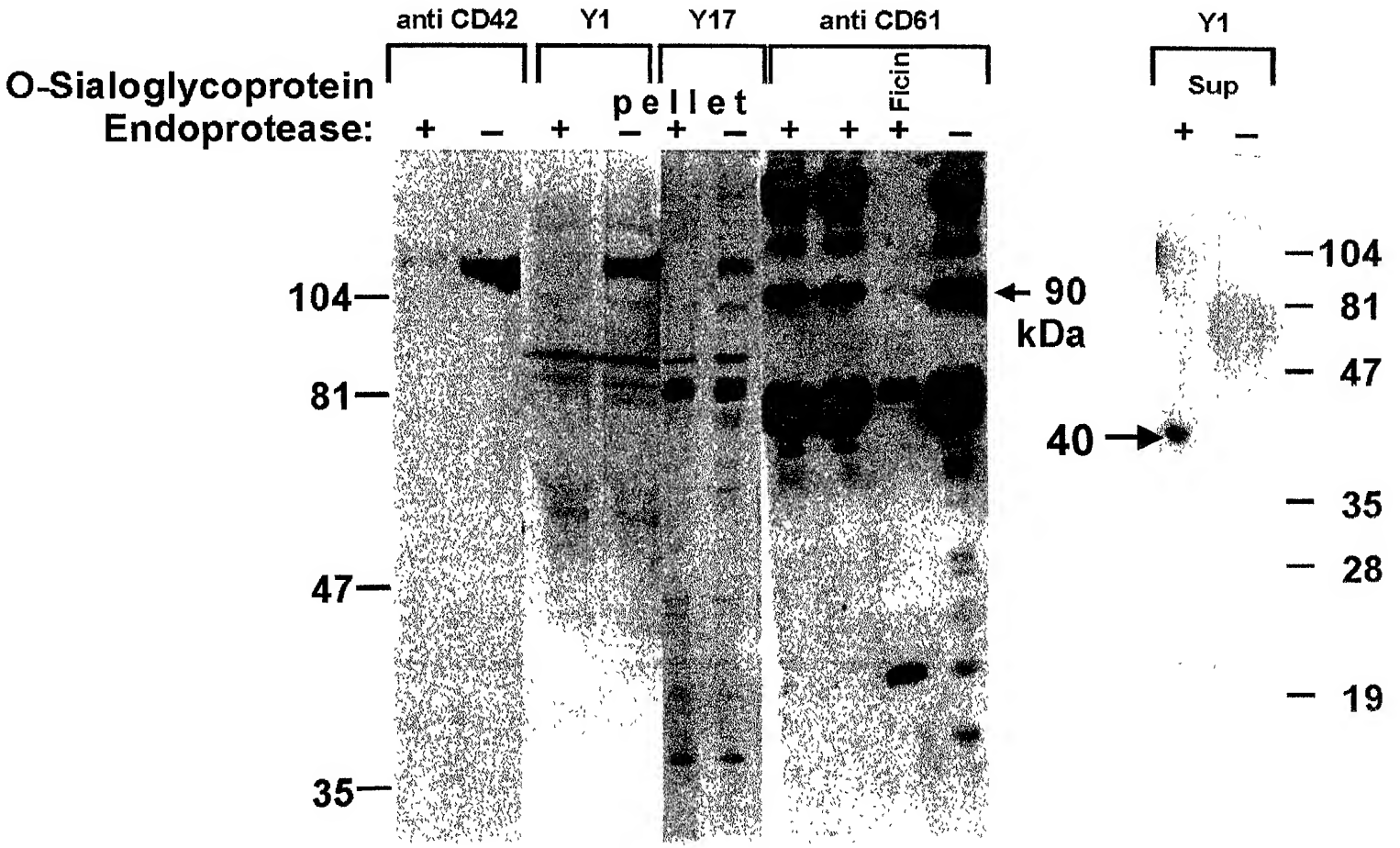


FIG. 4

Y1 and Y17 binds similar glycocalycin fragments after cleavage by O-Sialoglycoprotein Endoprotease

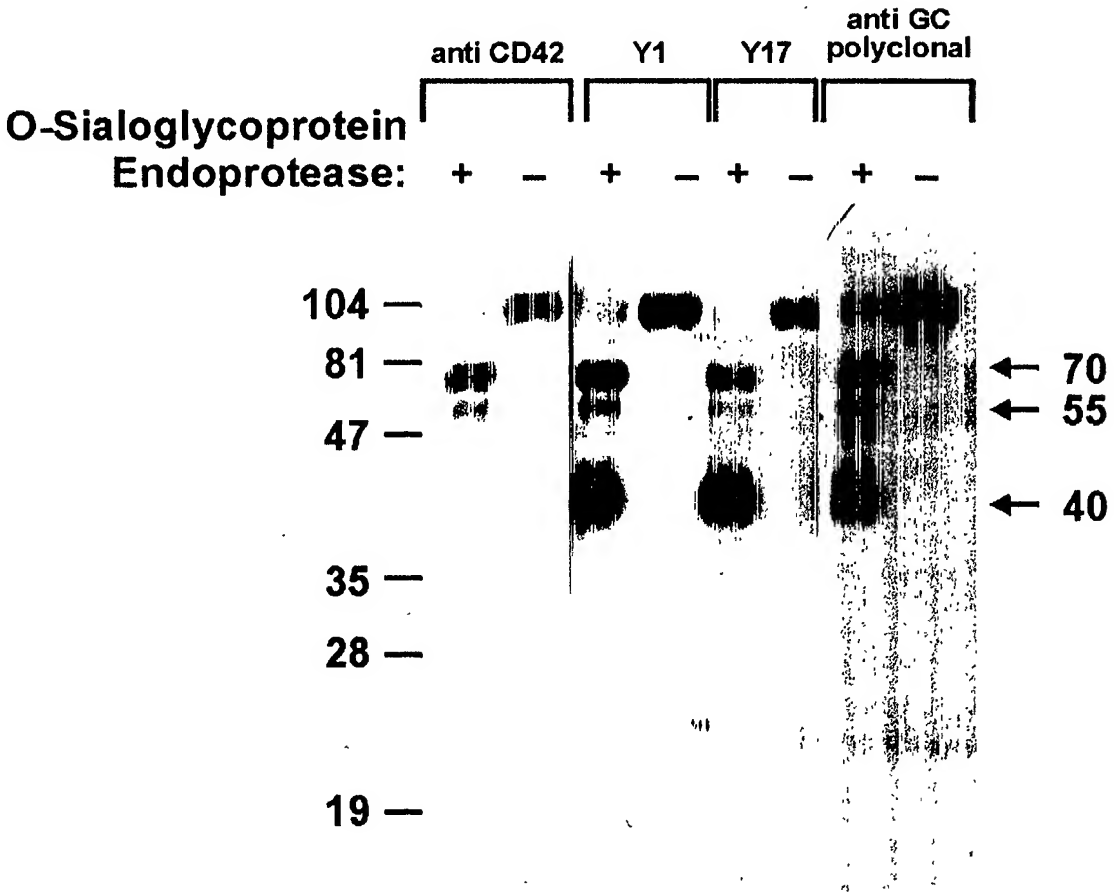
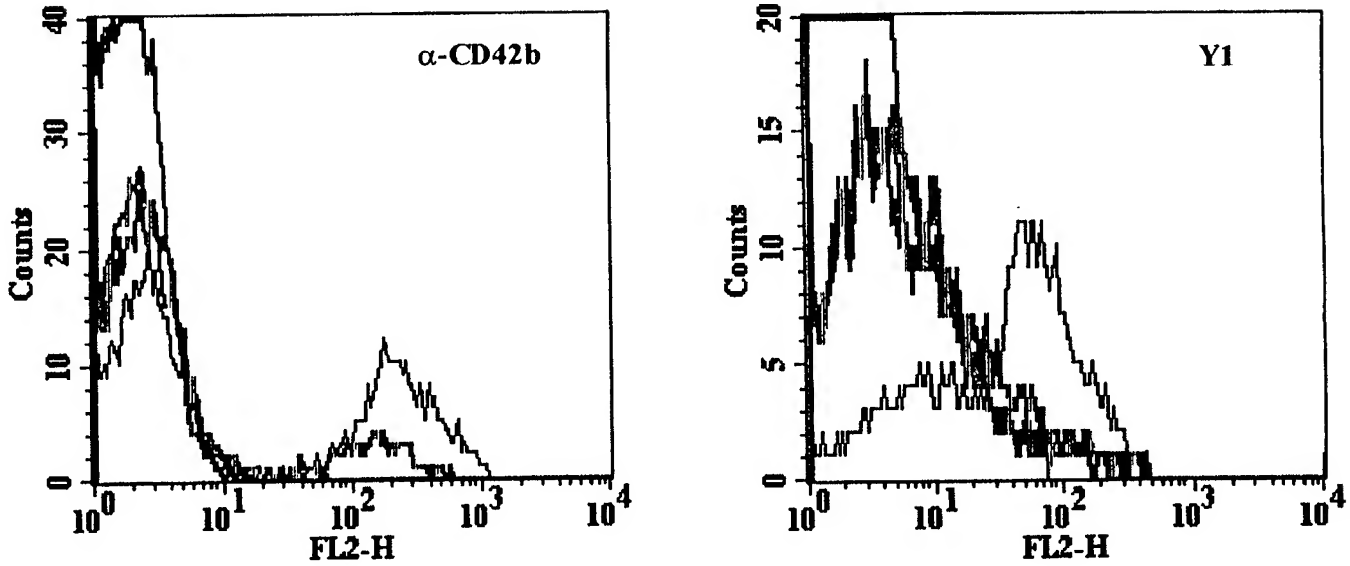


FIG. 5

Specific GPIb Proteolysis Abolishes Y1 Binding to Platelets



Key	Name	Parameter	G
	NON-TREATED PLATELETS		
—	O-SIALOGLYCOPROTEIN ENDO. (10 μg/ml)		
—	O-SIALOGLYCOPROTEIN ENDO. (50μ g/ml)		
—	FICIN (18 μg/ml)		

FIG. 6

Y1 binds N-terminal (His-1 - Glu 282) fragment of platelet GPIb after cleavage by mocarhagin

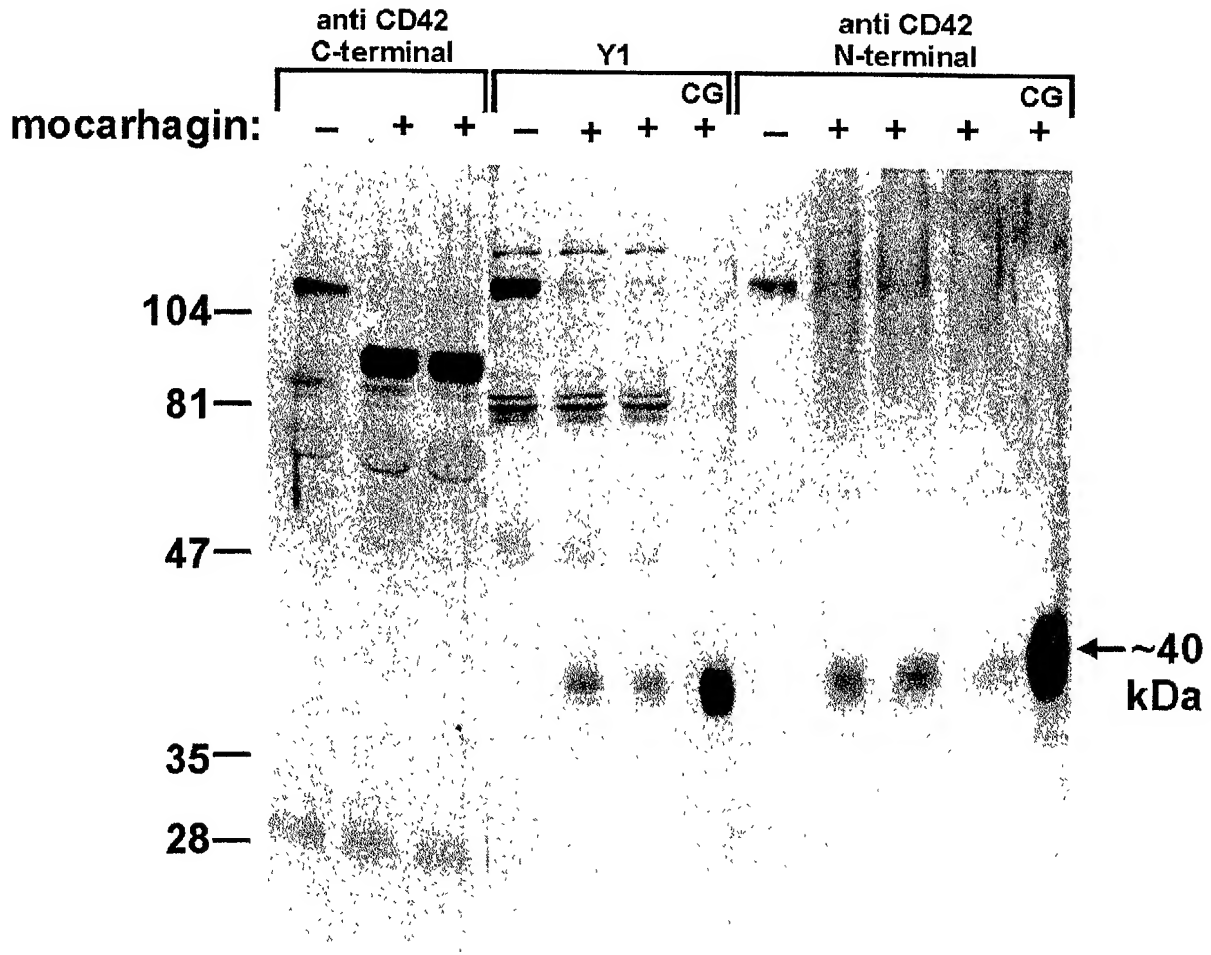


FIG. 7

Binding of Y1 and Y17 to glycocalycin after cleavage by mocarhagin

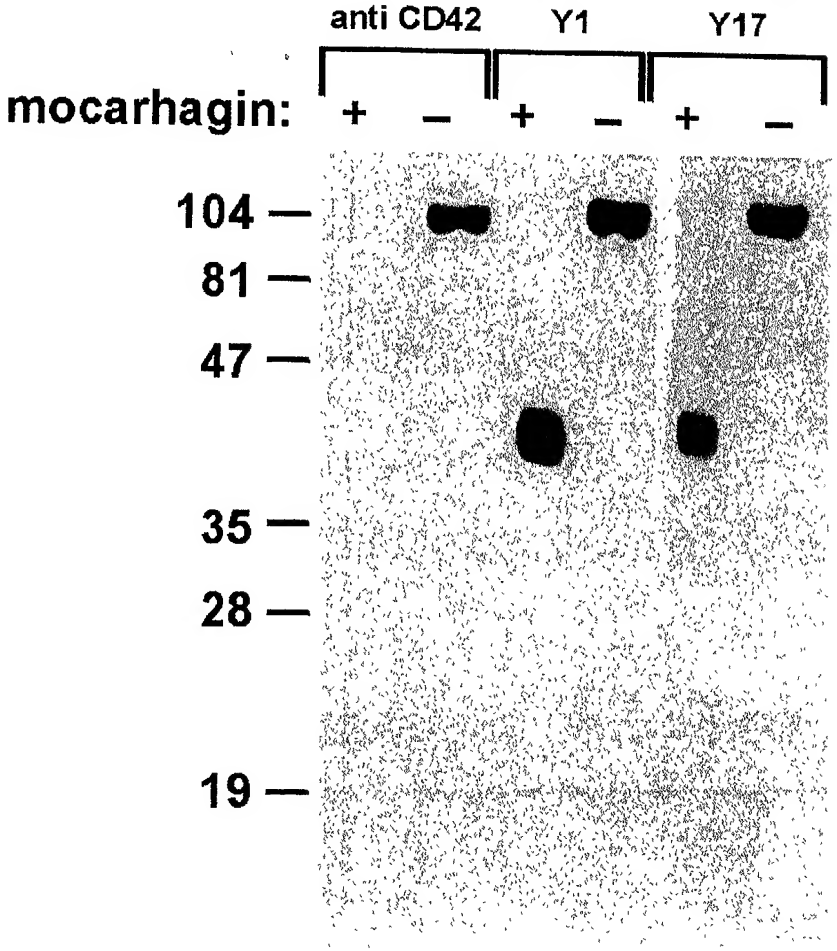
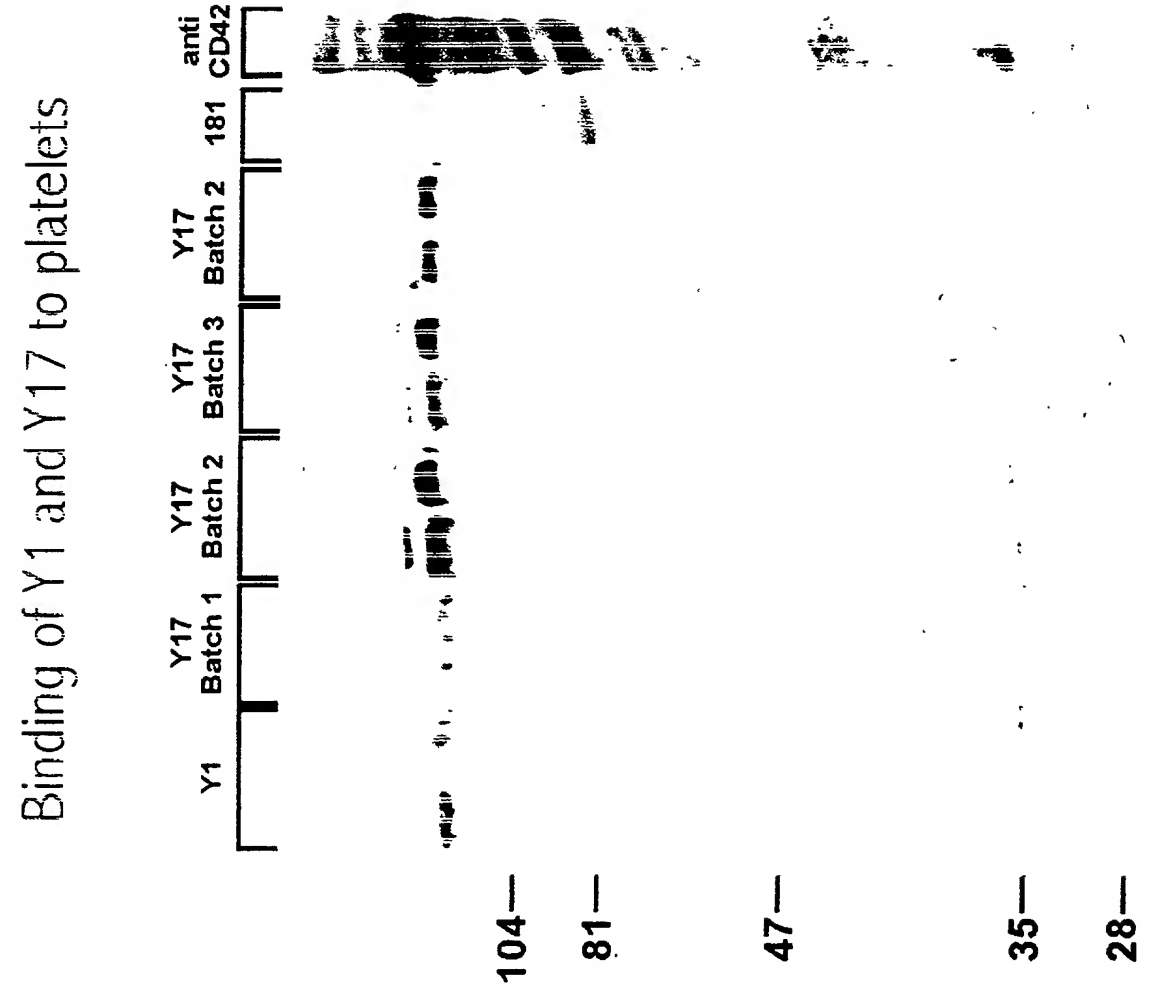


FIG. 8

FIG. 9


Binding of Y1 and Y17 to platelets
Y1
Y17 Batch 1
Y17 Batch 2
Y17 Batch 3
Y17 Batch 2
181
anti CD42
104
81
47
35
28

Y1 and Y17 bind glycocalycin similar after cleavage by Ficin

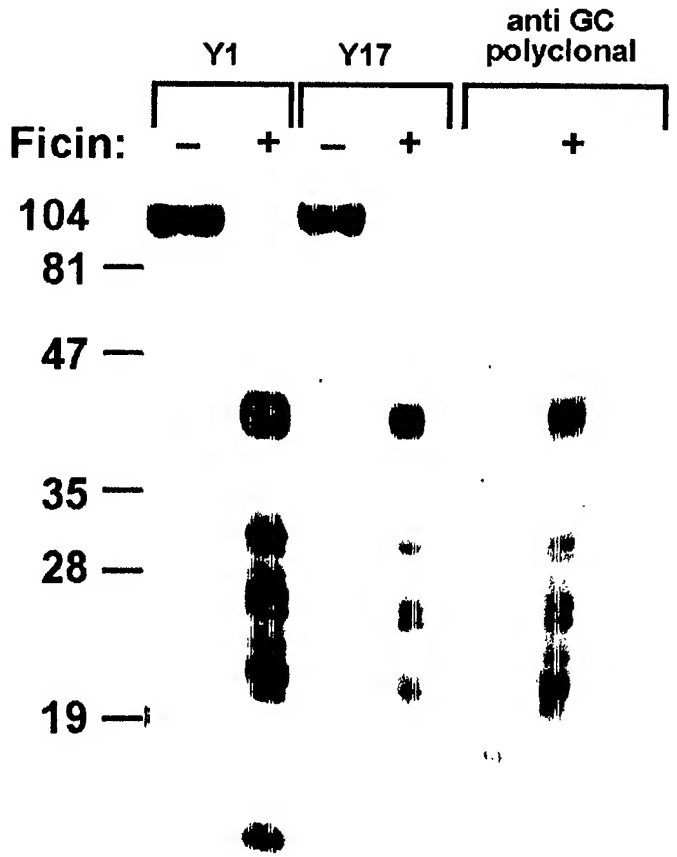
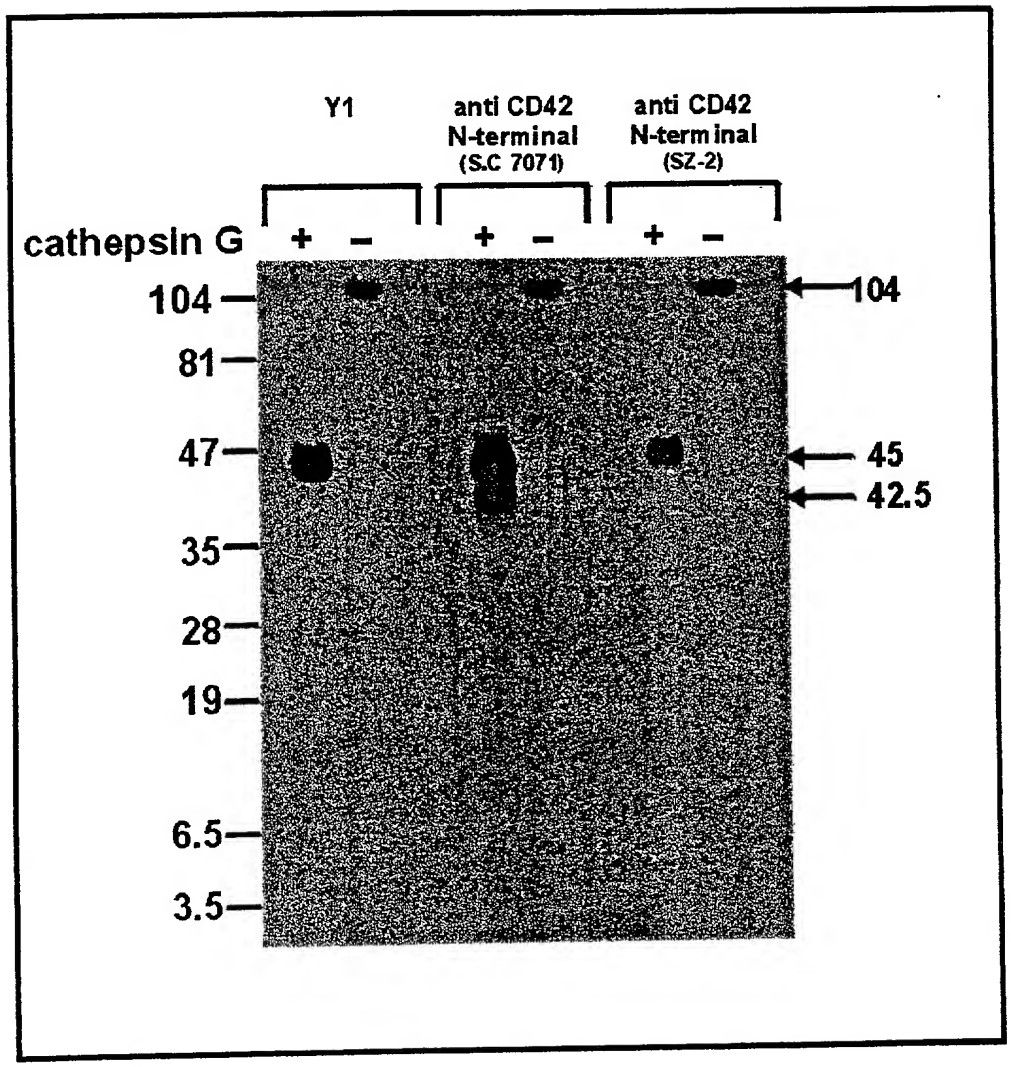


FIG. 10

FIG. 11


Y1
anti CD42
N-terminal
(S.C 7071)
anti CD42
N-terminal
(SZ-2)
cathepsin G
+ − + − + −
104
81
47
35
28
19
6.5
3.5
104
45
42.5

Y1 and Y17 reacts with larger cathepsin G cleaved platelets GPIb fragment

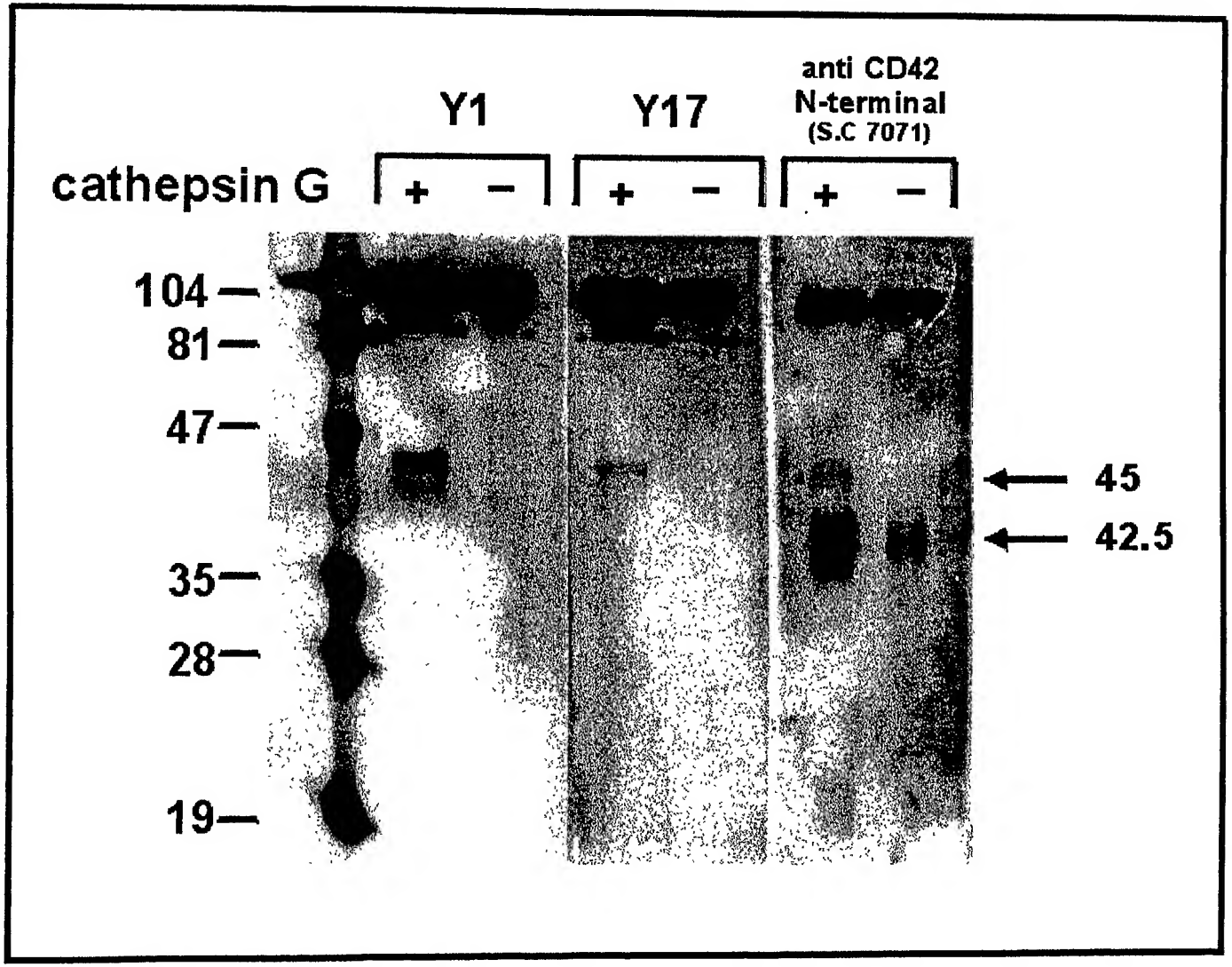
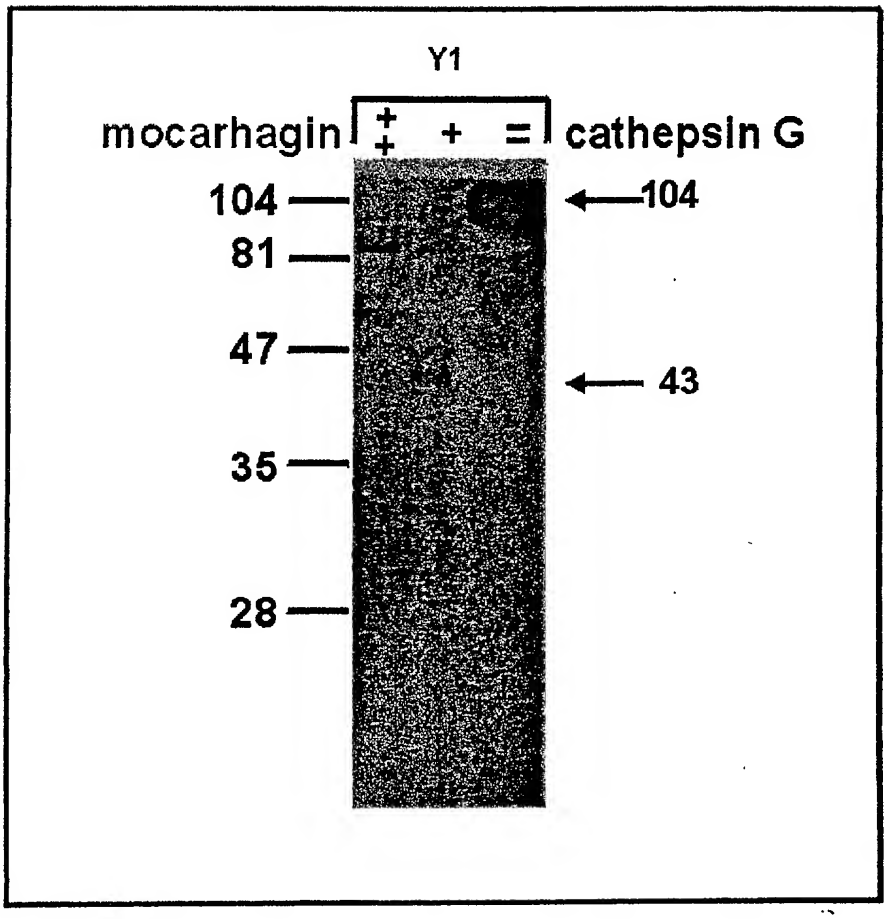


FIG. 12

FIG. 13



Cleavage of washed platelets by mocarhagin and cathepsin G

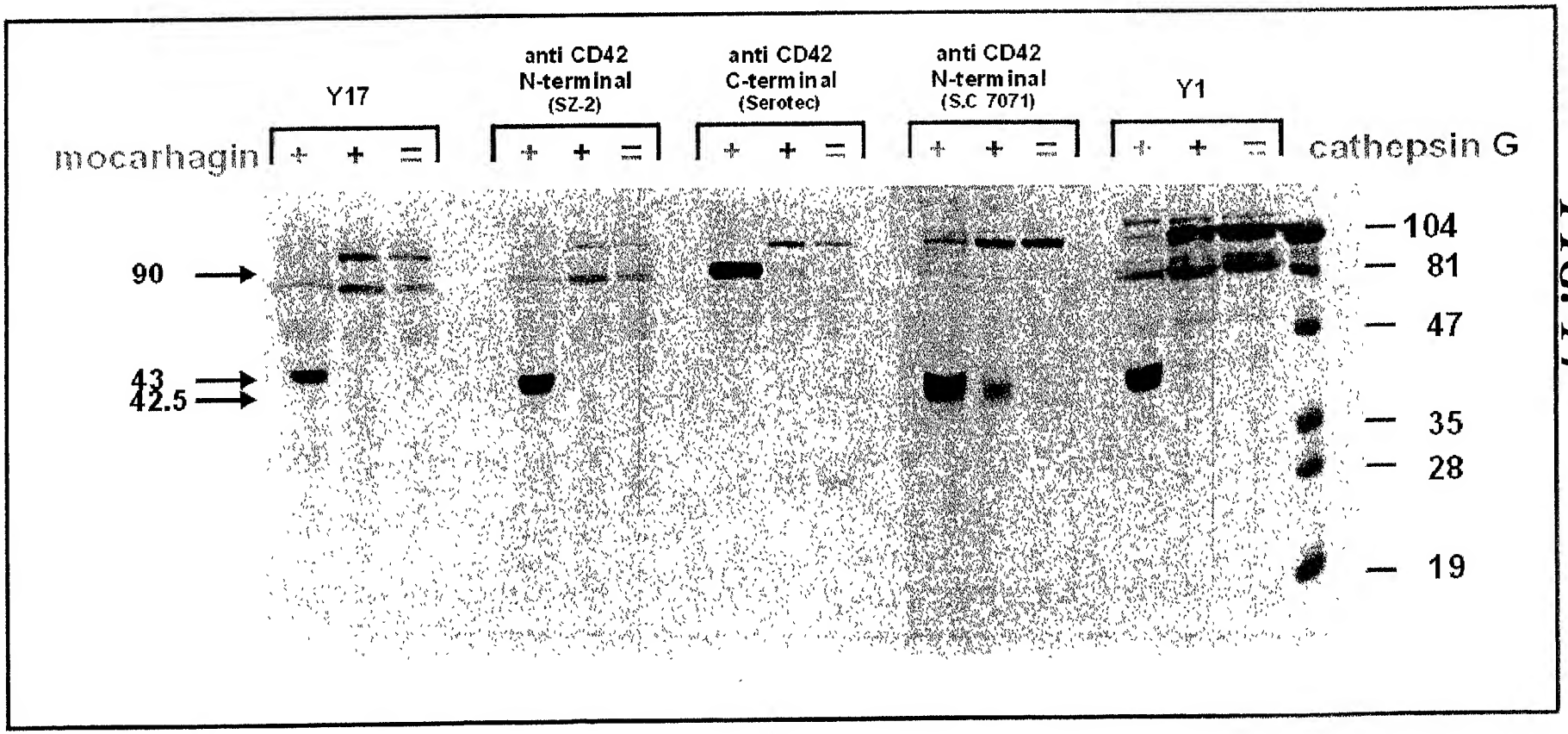


FIG. 14

FIG. 15

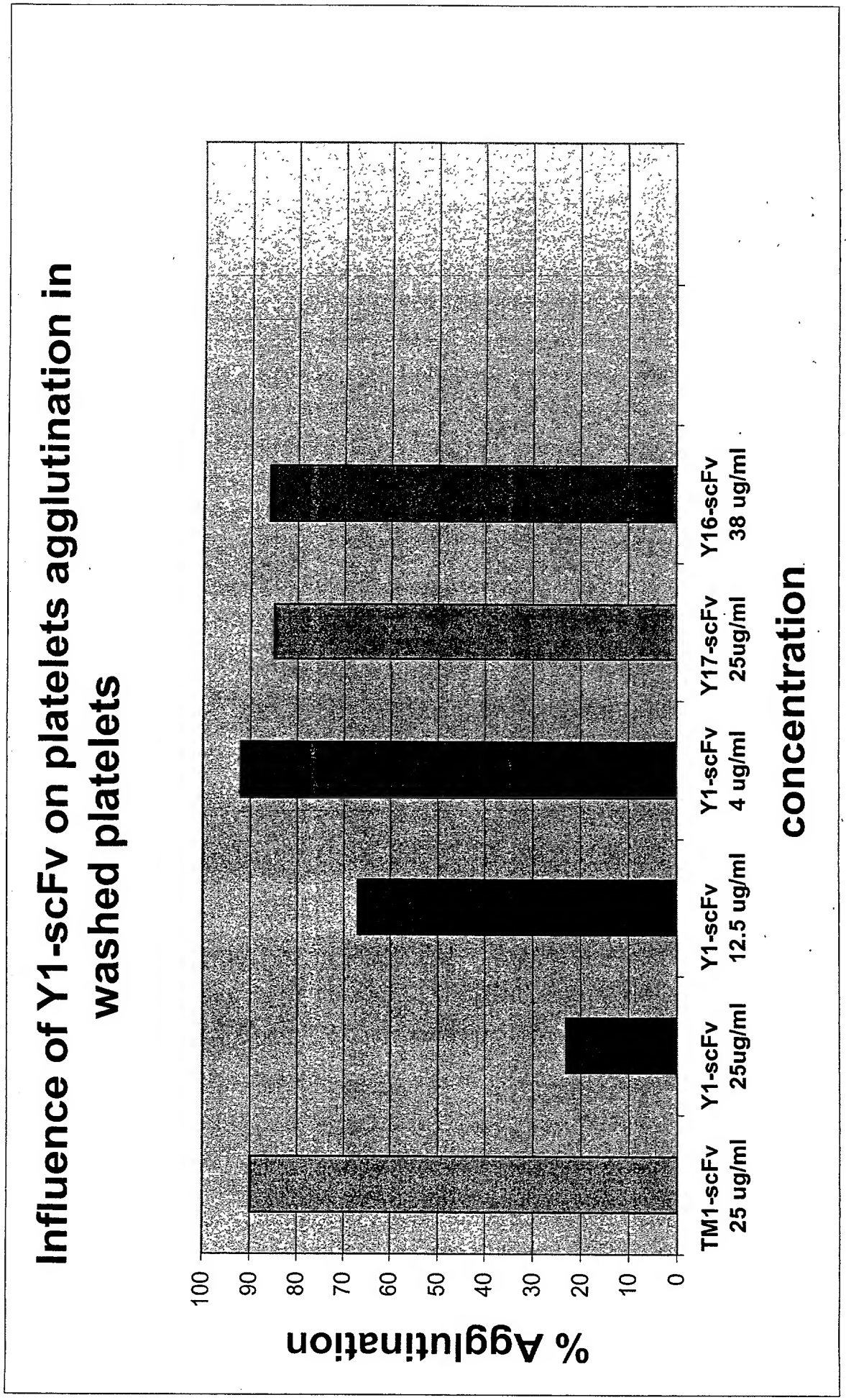

Influence of Y1-scFv on platelets agglutination in washed platelets
% Agglutination
100
90
80
70
60
50
40
30
20
10
0
TM1-scFv 25 ug/ml
Y1-scFv 25ug/ml
Y1-scFv 12.5 ug/ml
Y1-scFv 4 ug/ml
Y17-scFv 25ug/ml
Y16-scFv 38 ug/ml
concentration

FIG. 16

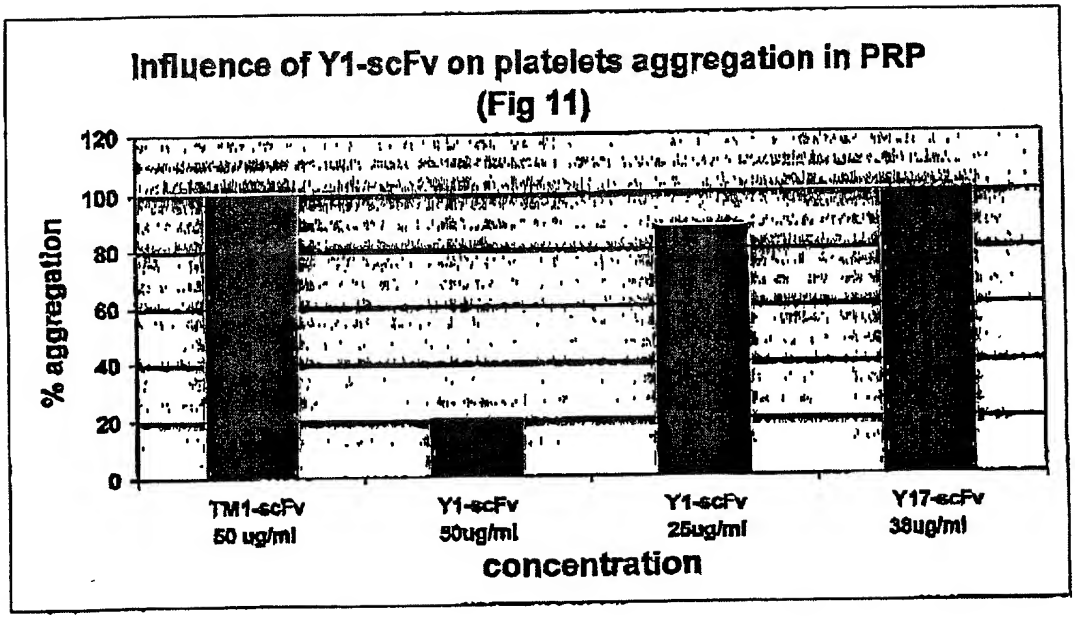

Influence of Y1-scFv on platelets aggregation in PRP
(Fig 11)
% aggregation
120
100
80
60
40
20
0
TM1-scFv
50 ug/ml
Y1-scFv
50ug/ml
Y1-scFv
25ug/ml
Y17-scFv
38ug/ml
concentration

FIG. 17

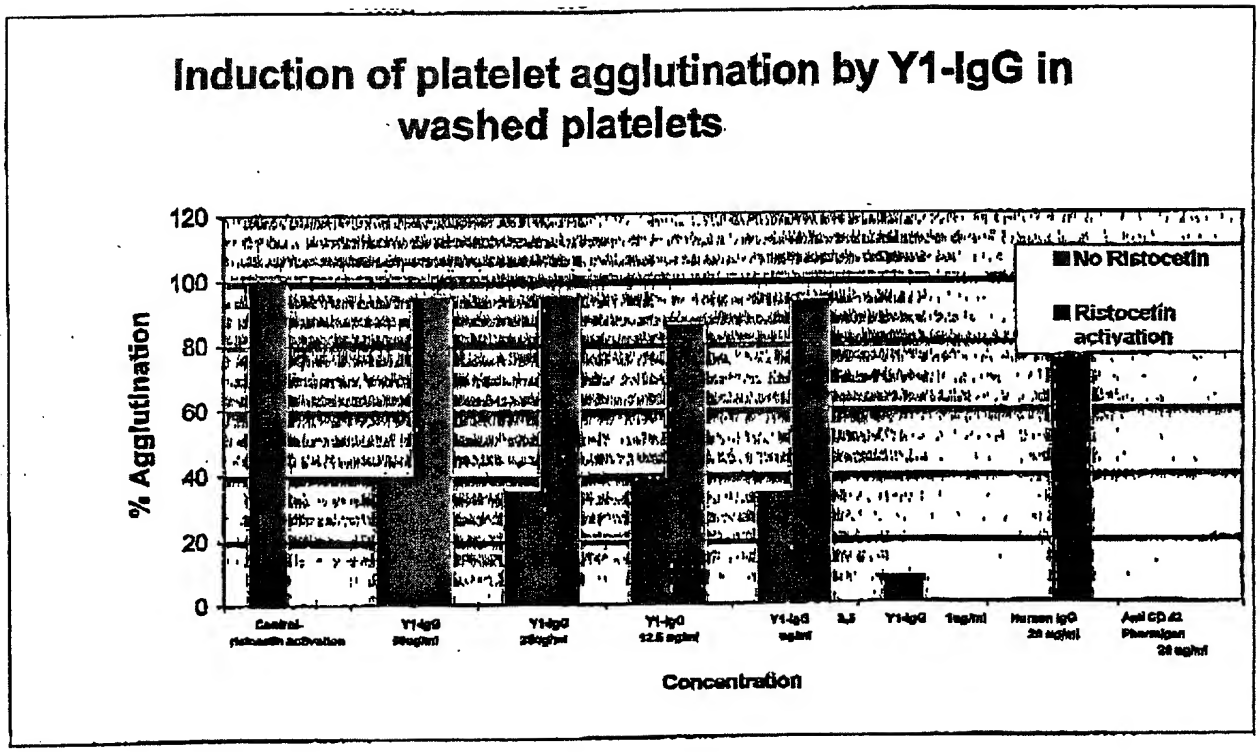

Induction of platelet agglutination by Y1-IgG in washed platelets
% Agglutination
120
100
80
60
40
20
0
No Ristocetin
Ristocetin activation
Concentration

FIG. 18

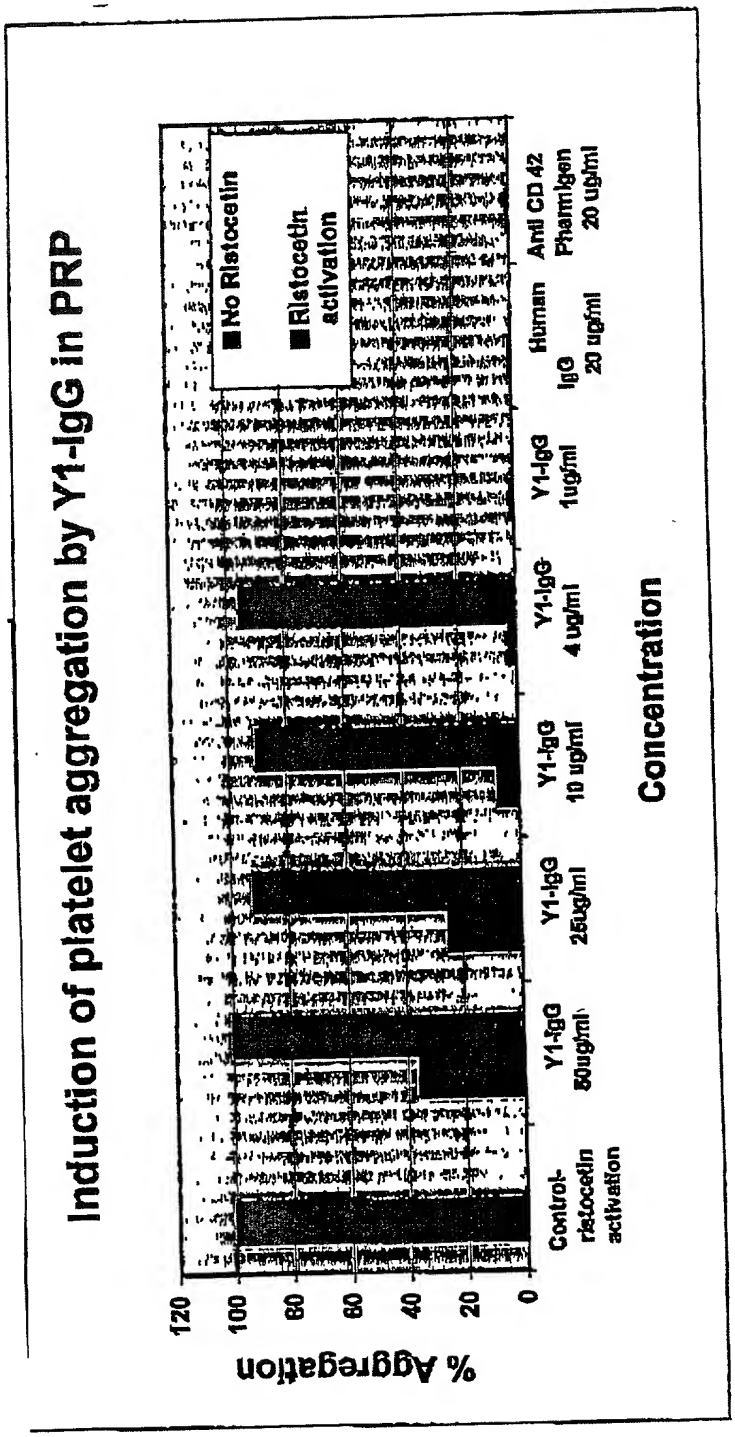

Induction of platelet aggregation by Y1-IgG in PRP
No Ristocetin
Ristocetin activation
% Aggregation
120
100
80
60
40
20
0
Control-ristocetin activation
Y1-IgG 50ug/ml
Y1-IgG 25ug/ml
Y1-IgG 10 ug/ml
Y1-IgG 4 ug/ml
Y1-IgG 1ug/ml
Human IgG 20 ug/ml
Anti CD 42 Phamigen 20 ug/ml
Concentration

FIG. 19

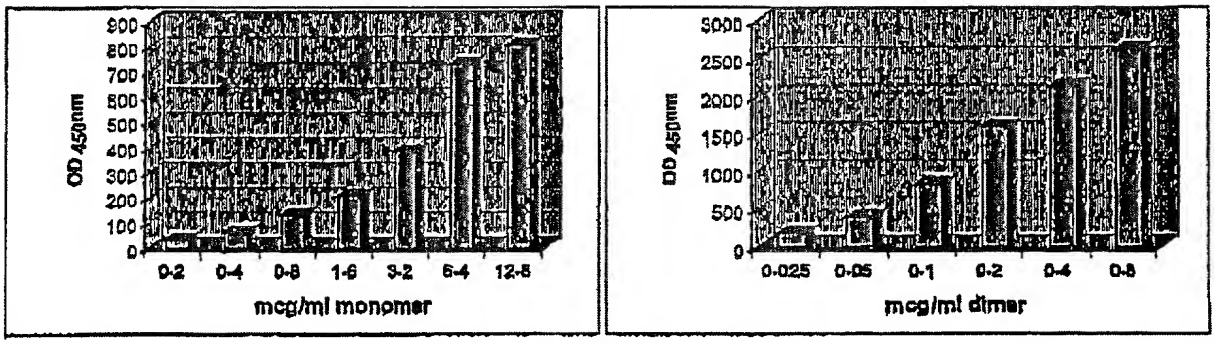
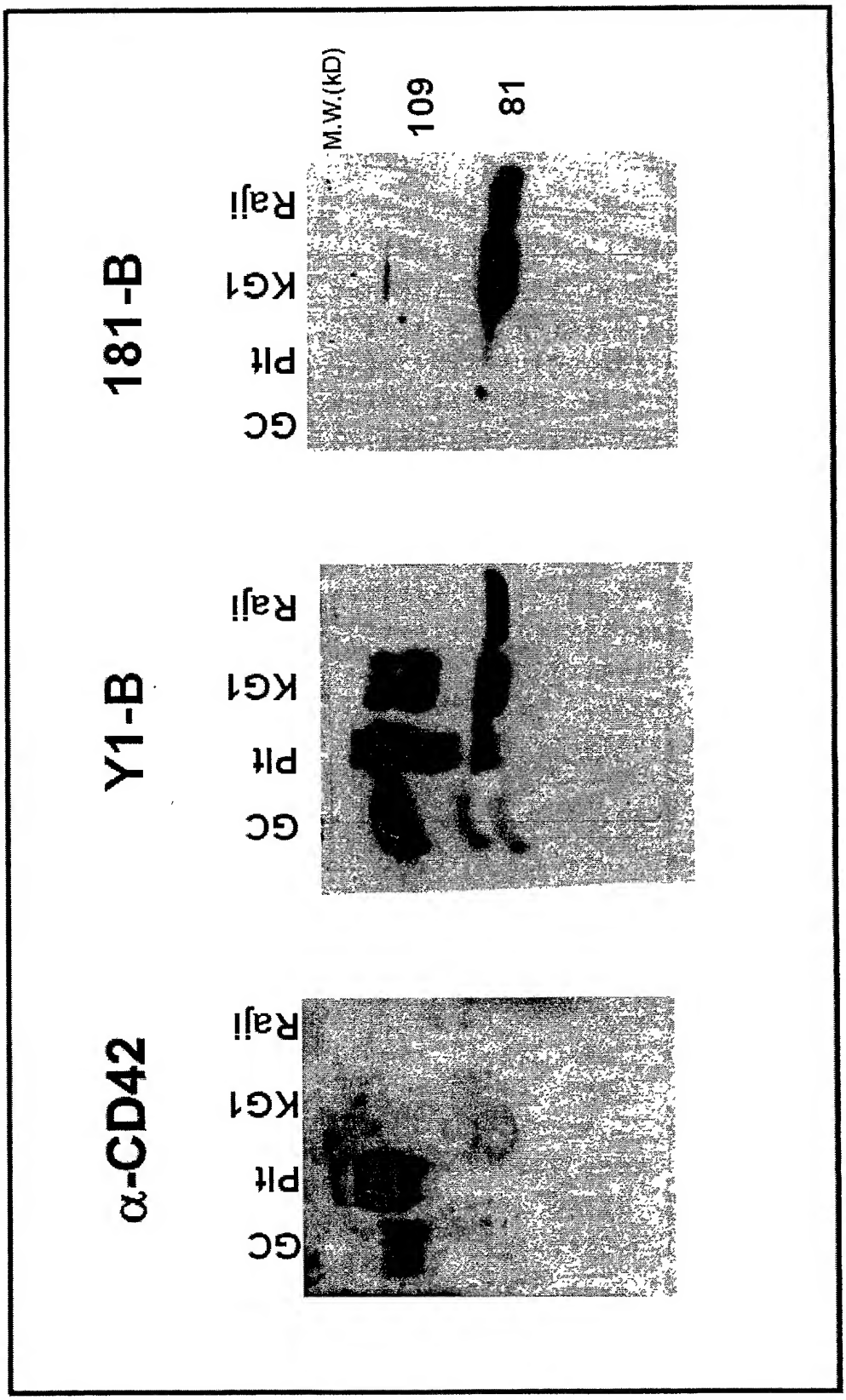

OD 450nm
900
800
700
600
500
400
300
200
100
0
0-2
0-4
0-8
1-6
3-2
6-4
12-8
mcg/ml monomer
OD 450nm
3000
2500
2000
1500
1000
500
0
0-025
0-05
0-1
0-2
0-4
0-8
mcg/ml dimer

FIG. 20


Specificity of Binding of Y1
and α-CD42 (N1-19)
to their Ligands
α-CD42
Y1-B
181-B
GC
Plt
KG1
Raji
M.W.(kD)
109
81

Y1-Ligand from KG1 membranes following Immuno-Precipitation with Y1: Purification on RP-HPLC

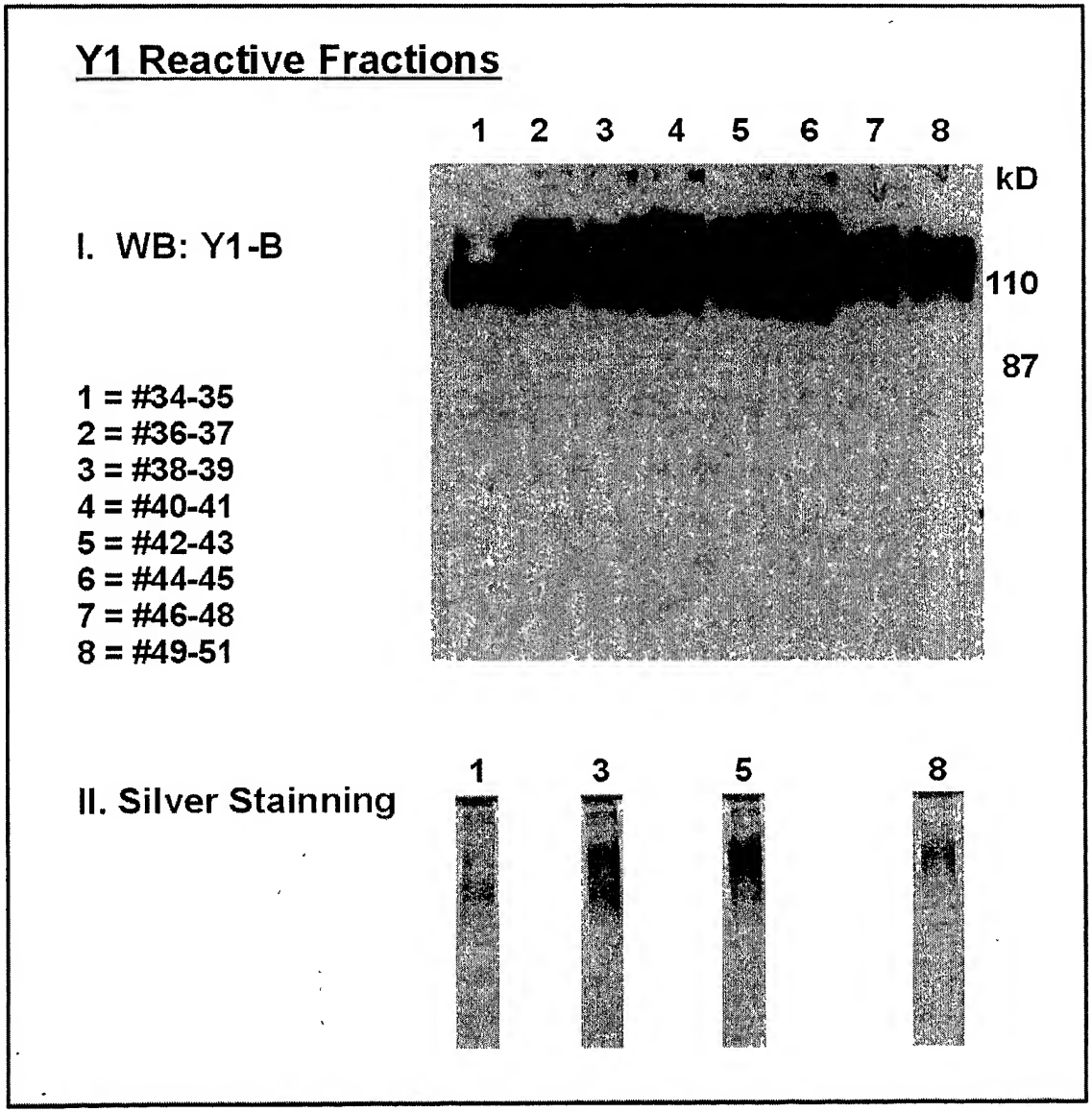


FIG. 21

Effect of O-Sialo-Glycoprotein Endopeptidase on Y1 Binding

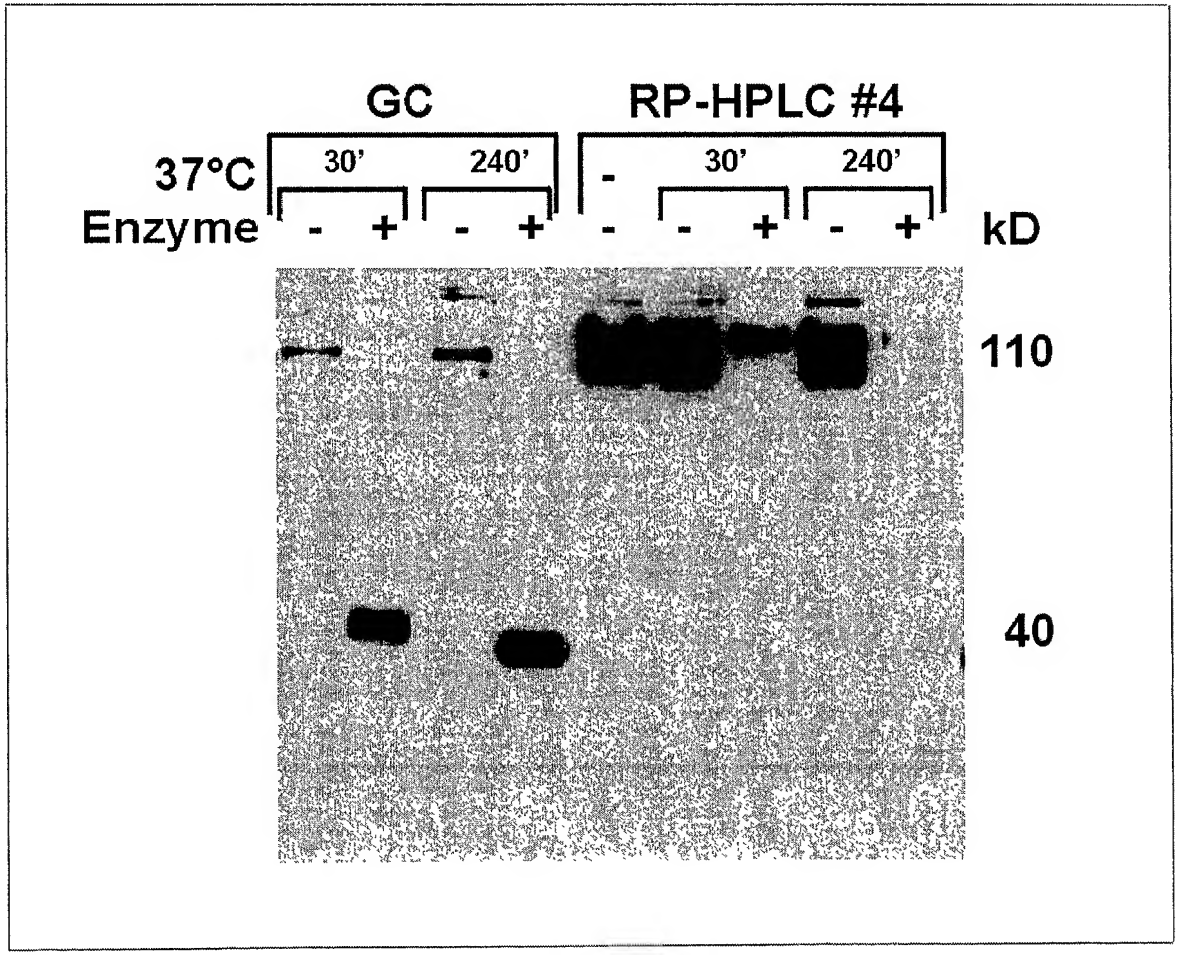


FIG. 22

Effect of Aryl-Sulfatase on Binding of Y1: RP-HPLC(KG1) & H-B(Heparin-BSA)

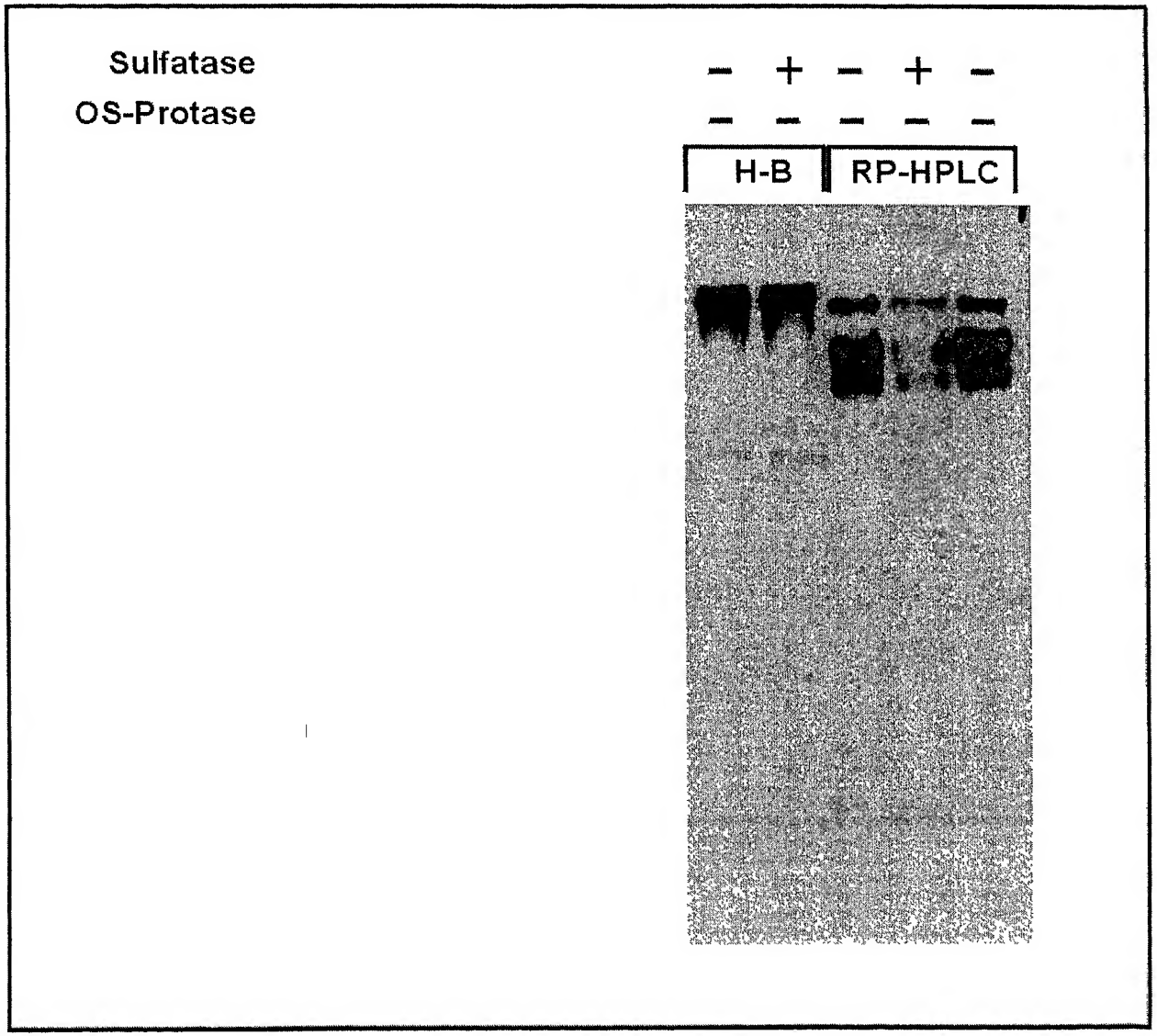
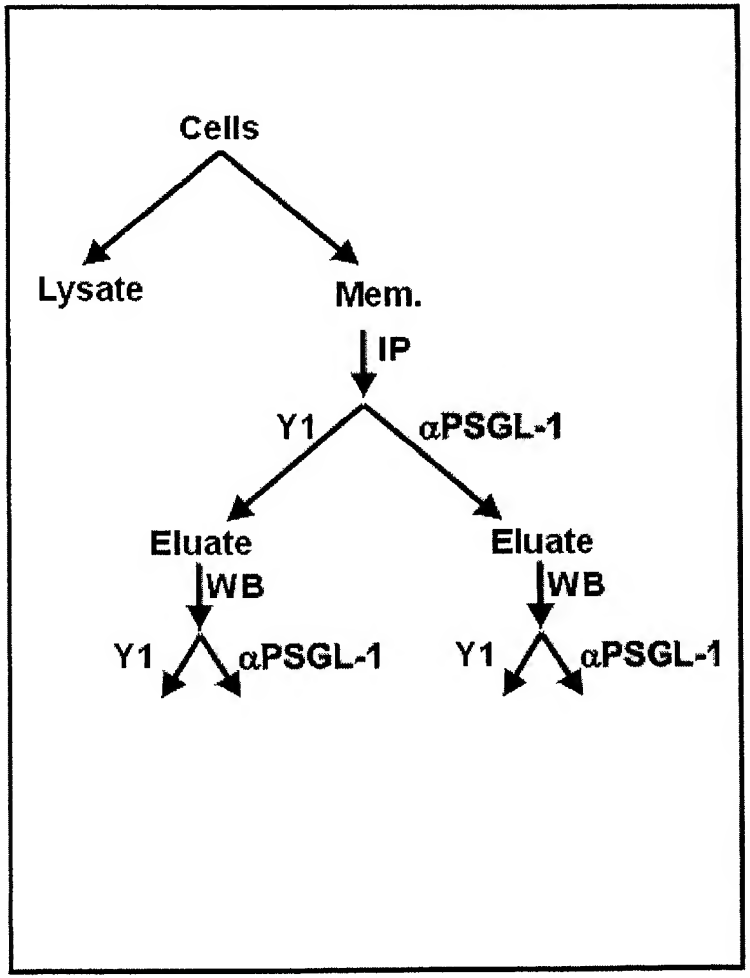


FIG. 23

Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1



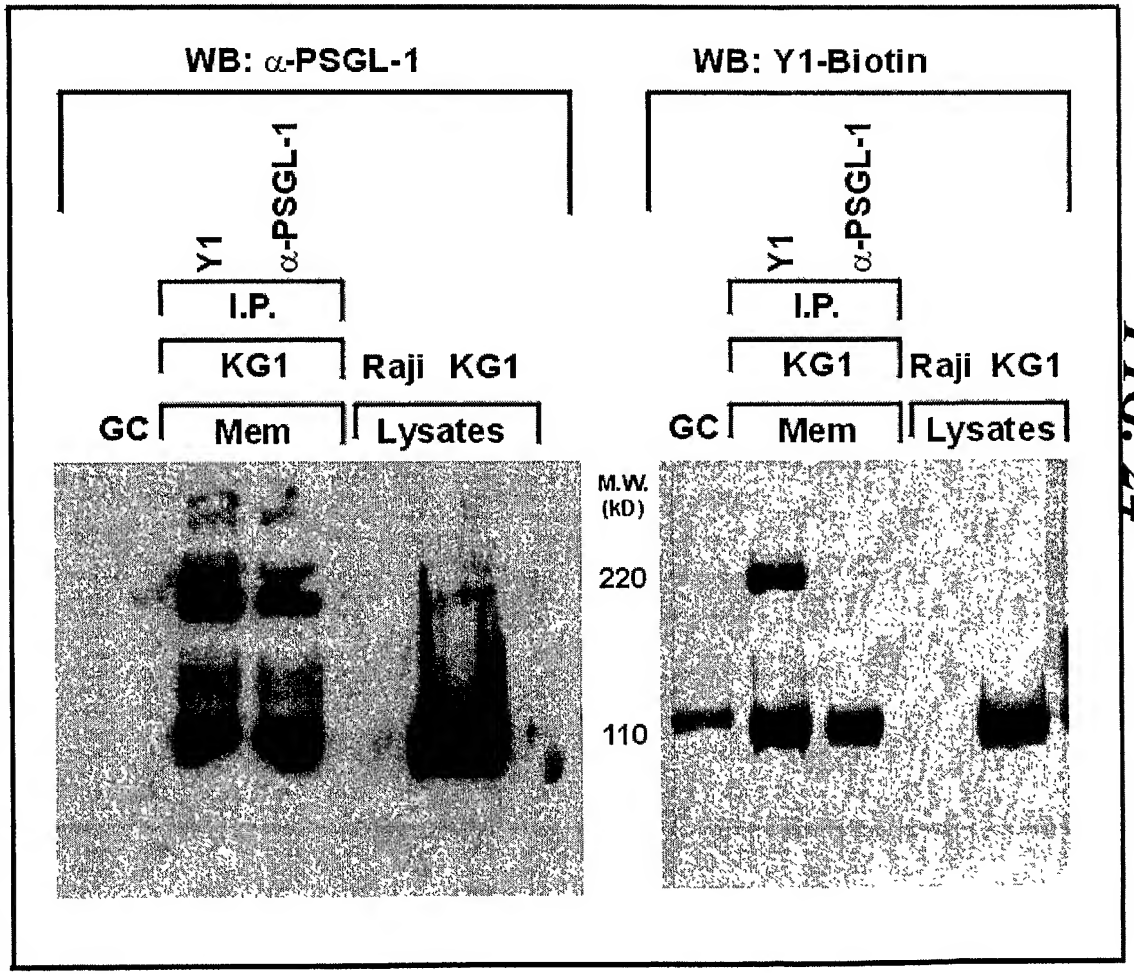
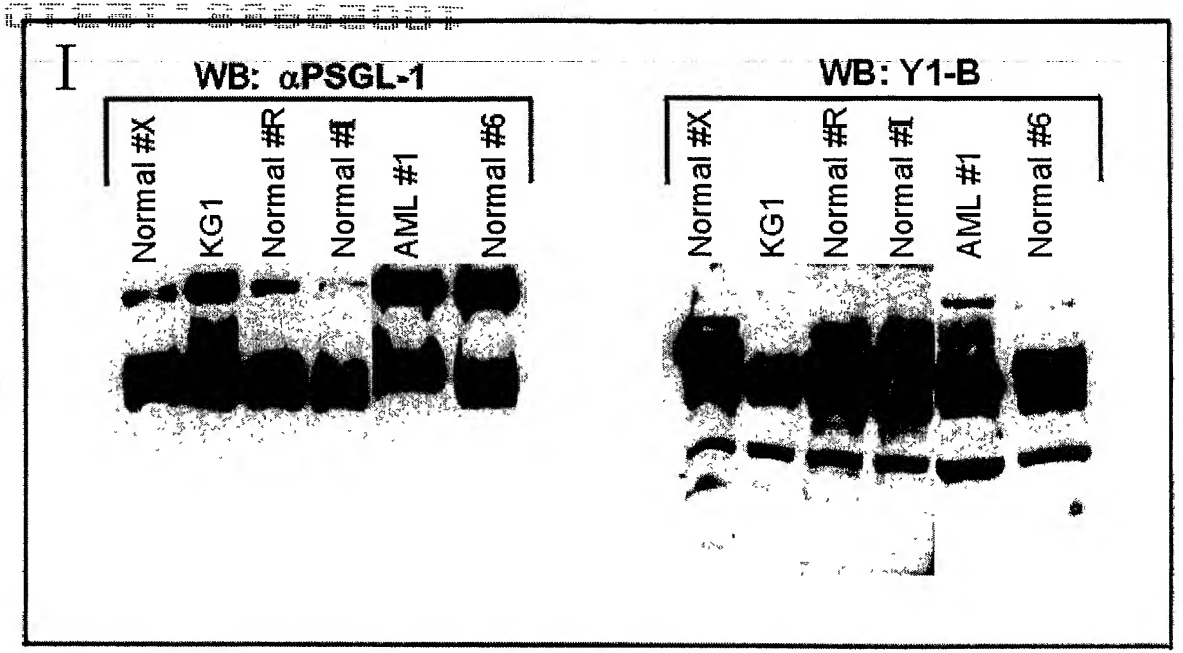
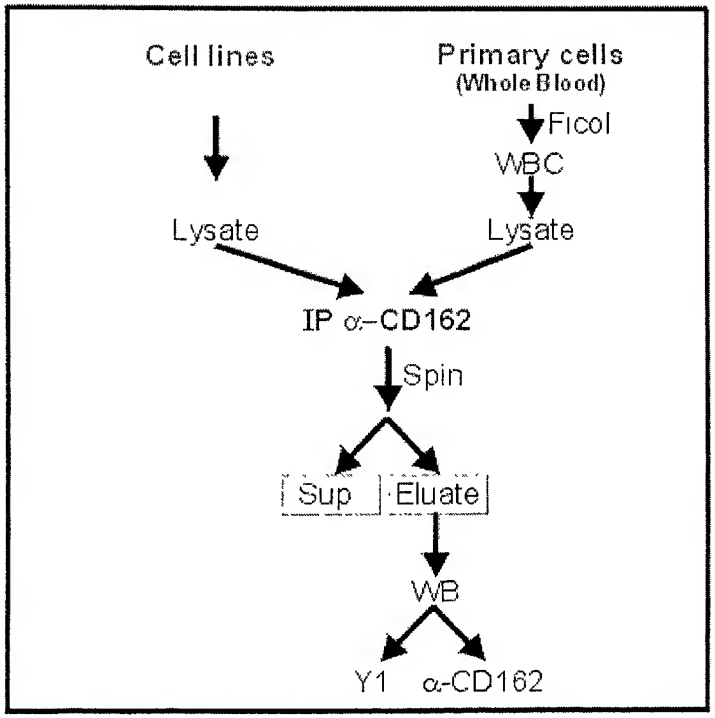


FIG. 24

α-CD162 and Y1:
Comparison between cells from AML patient and normal blood





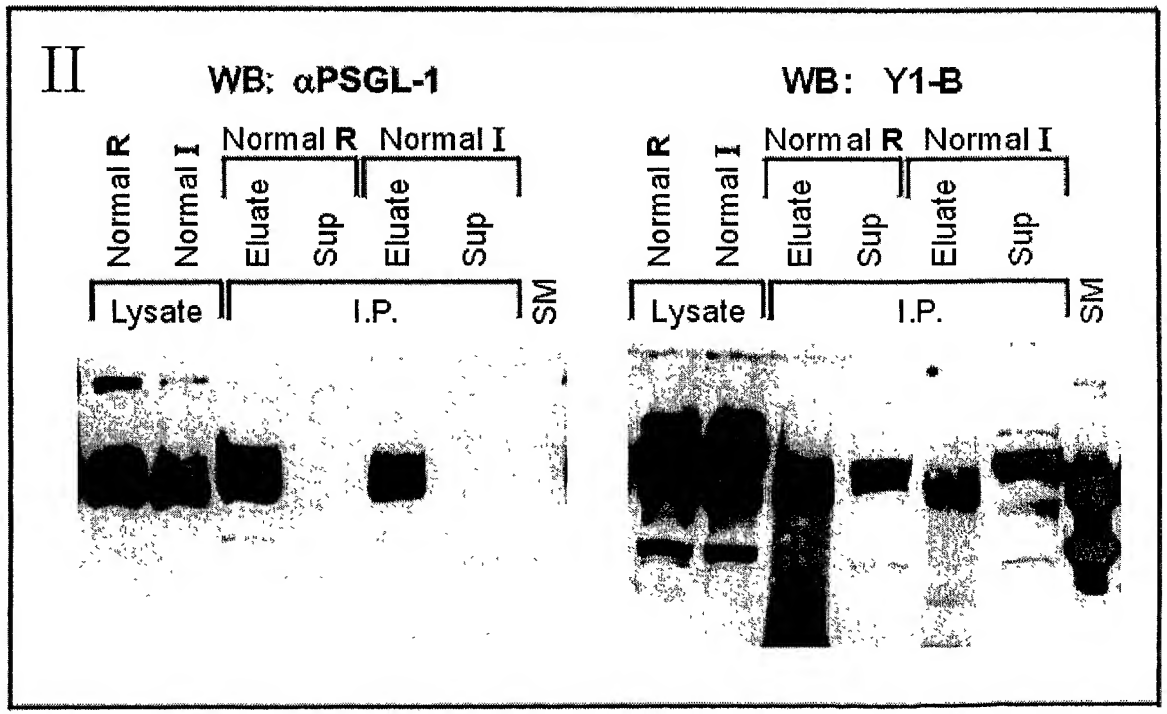
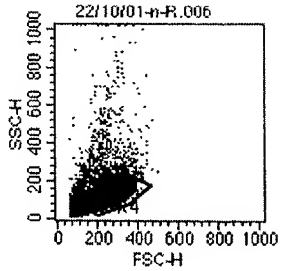
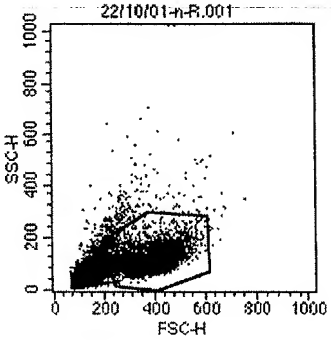
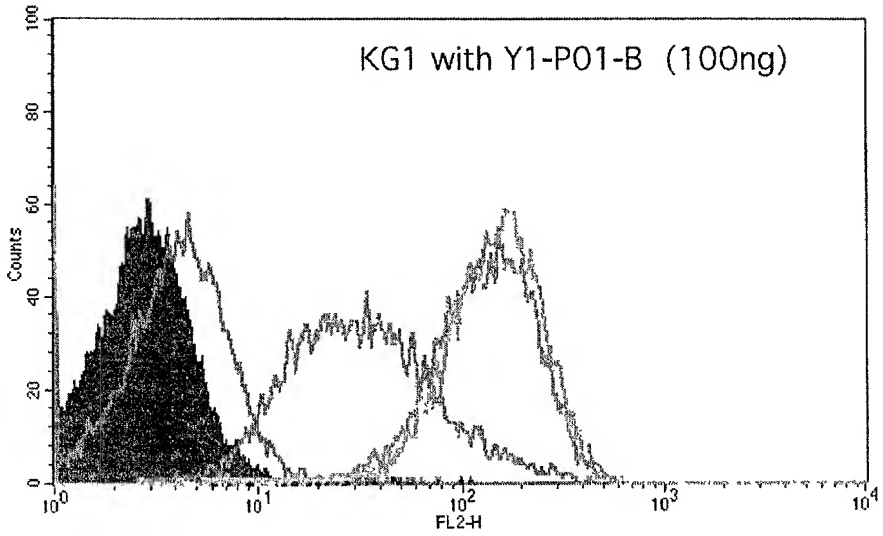
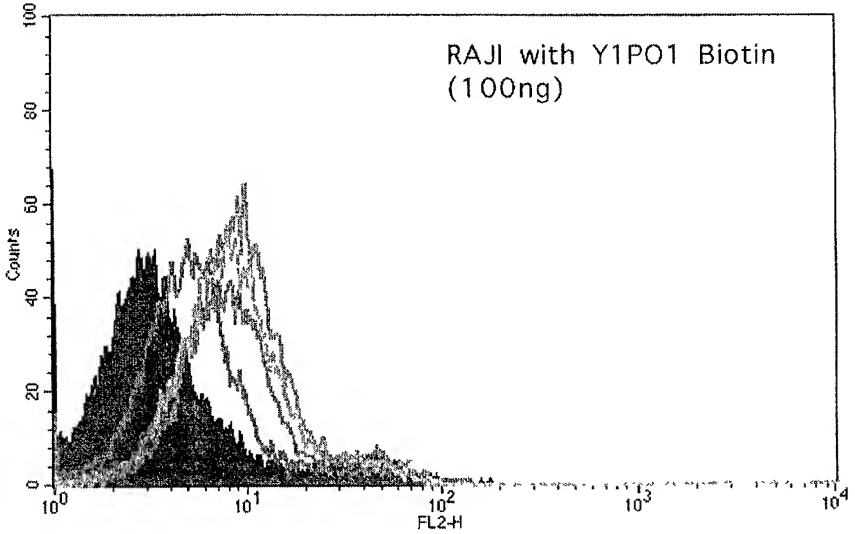


FIG. 25







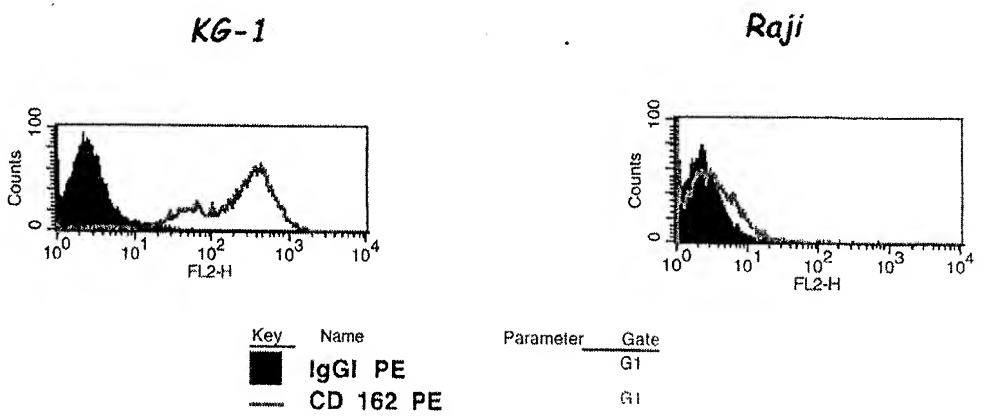


Key	Name	Parameter	Gate
	22/10/01-n-R 006	N01-B	
	22/10/01-n-R 007	P01-B	
	22/10/01-n-R 008	+KPL1	
	22/10/01-n-R 00	+PL1	
		+PL2	

FIG. 26

Specificity of Y1 Binding: Analysis by FACS

- Binding of α PSGL1 (αCD162/KPL1); competition with Y1-IgG



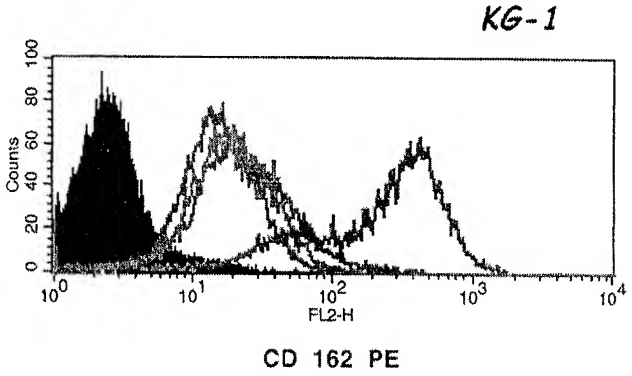
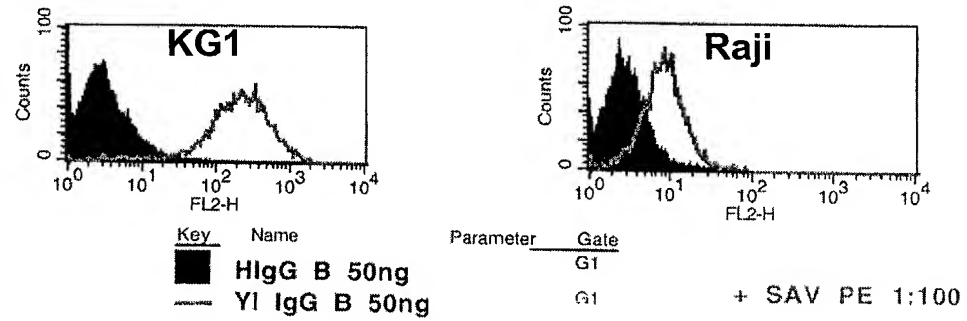


FIG. 27

Specificity of Y1 Binding: Analysis by FACS

- Binding of Y1-IgG; competition with αPSGL-1 (CD162 /KPL1)



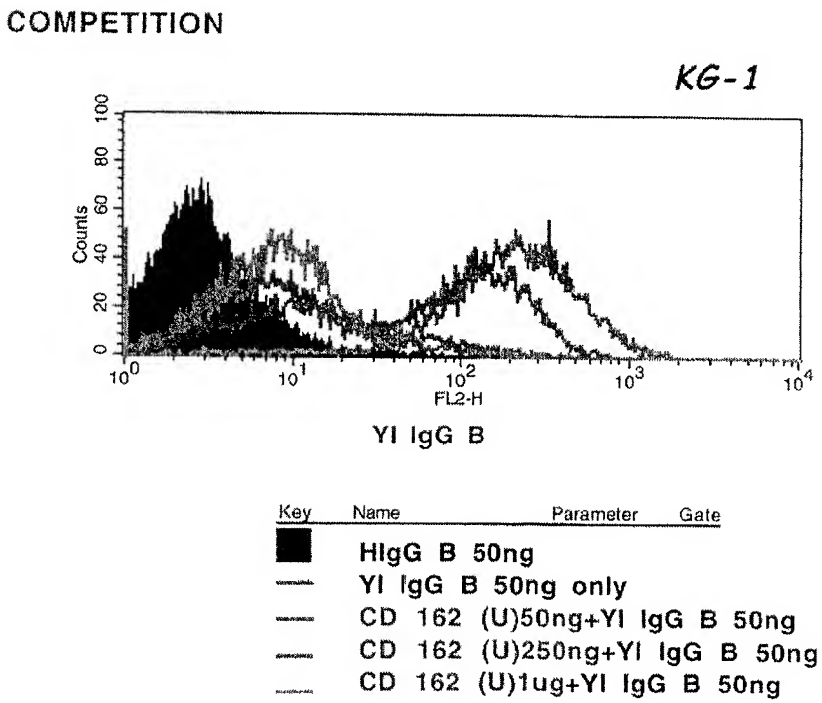


FIG. 28

FIG. 29

	F-YY	F-SS	P-YYY	P-SYY	P-SSY	P-SYS	P-YSY	P-YSS	P-YYS	P-SSS	G-YYY	G-SSS
100µM	0	0	18	83	96	100	85	100	100	100	28	100
10µM	13	22	20	31	50	100	48	100	100	95	37	75
1µM	0	16	23	22	16	65	42	91	91	60	30	36

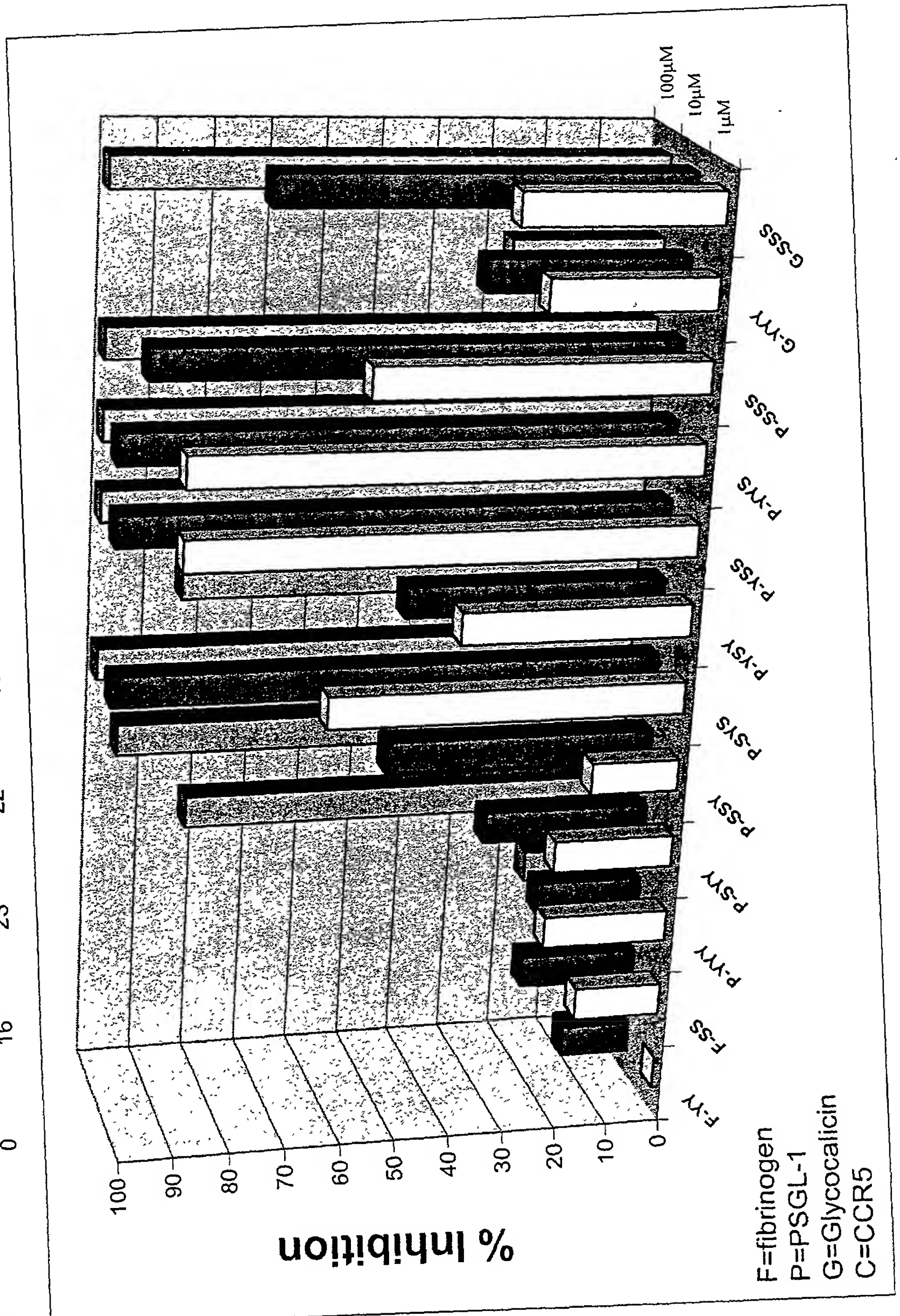


FIG. 30

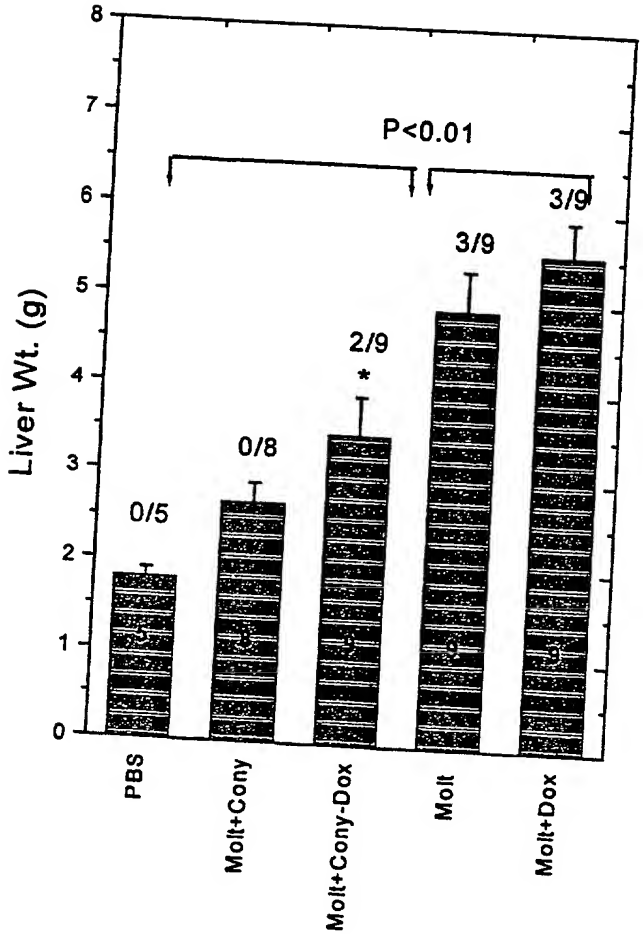

Liver Wt. (g)
0
1
2
3
4
5
6
7
8
P<0.01
0/5
0/8
2/9
*
3/9
3/9
PBS
Molt+Cony
Molt+Cony-Dox
Molt
Molt+Dox

FIG. 31

*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.

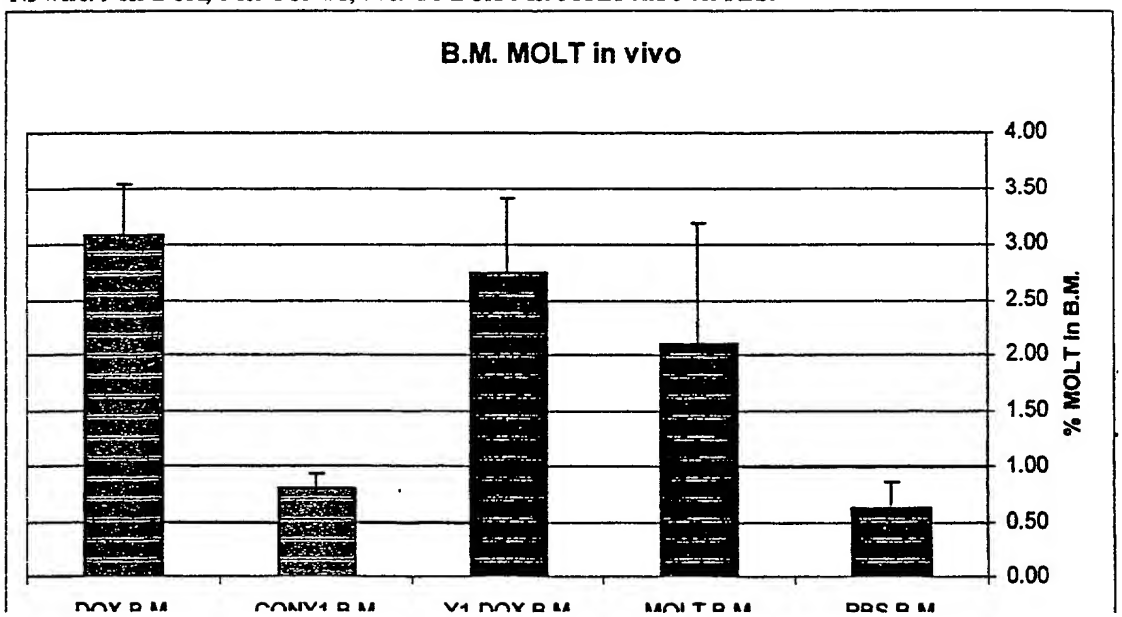
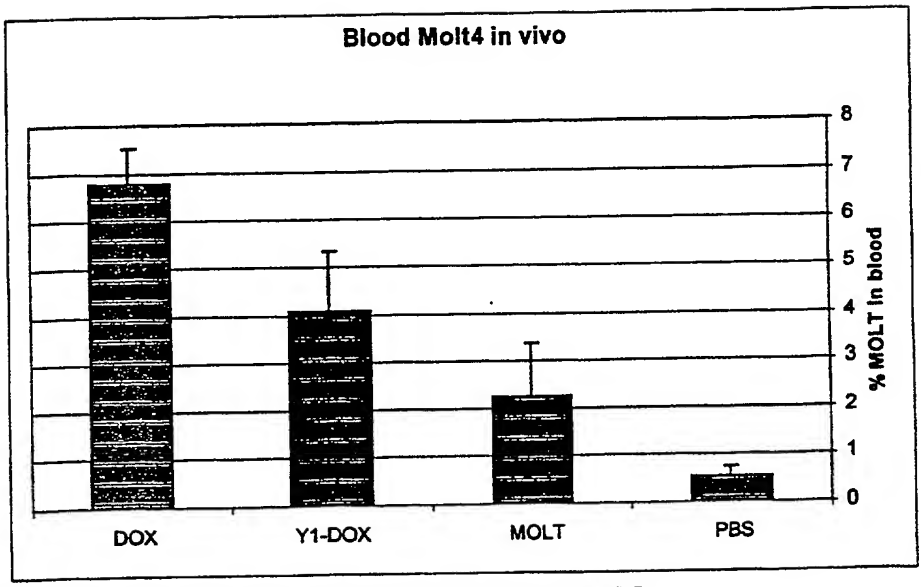


FIG. 32



**Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.

FIG. 33

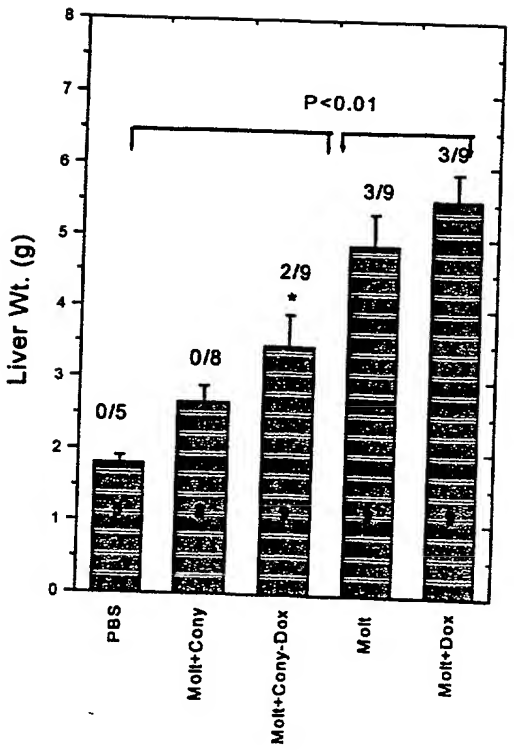

Liver Wt. (g)
8
7
6
5
4
3
2
1
0
P<0.01
0/5
0/8
2/9
*
3/9
3/9
PBS
Molt+Cony
Molt+Cony-Dox
Molt
Molt+Dox

FIG. 34

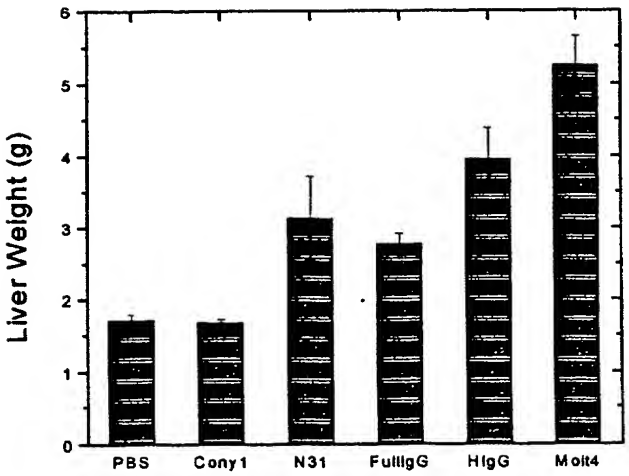


FIG. 35

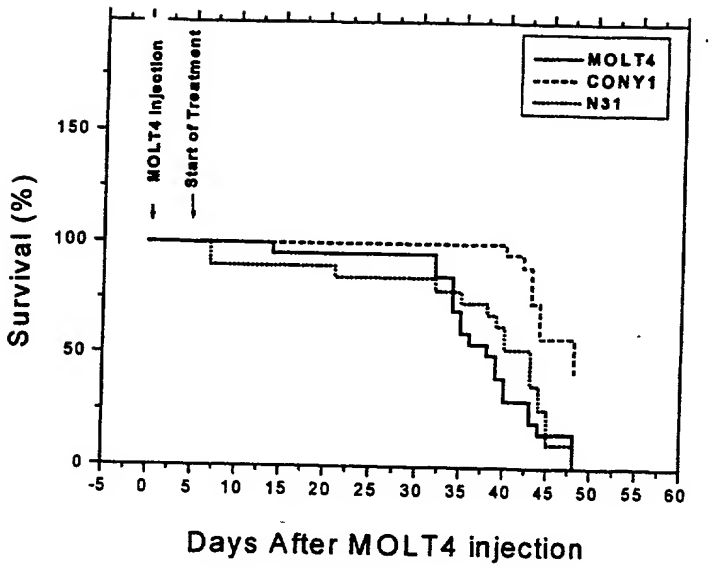

MOLT4
CONY1
N31
MOLT4 injection
Start of Treatment
Survival (%)
150
100
50
0
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
Days After MOLT4 injection

FIG. 36

***Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.

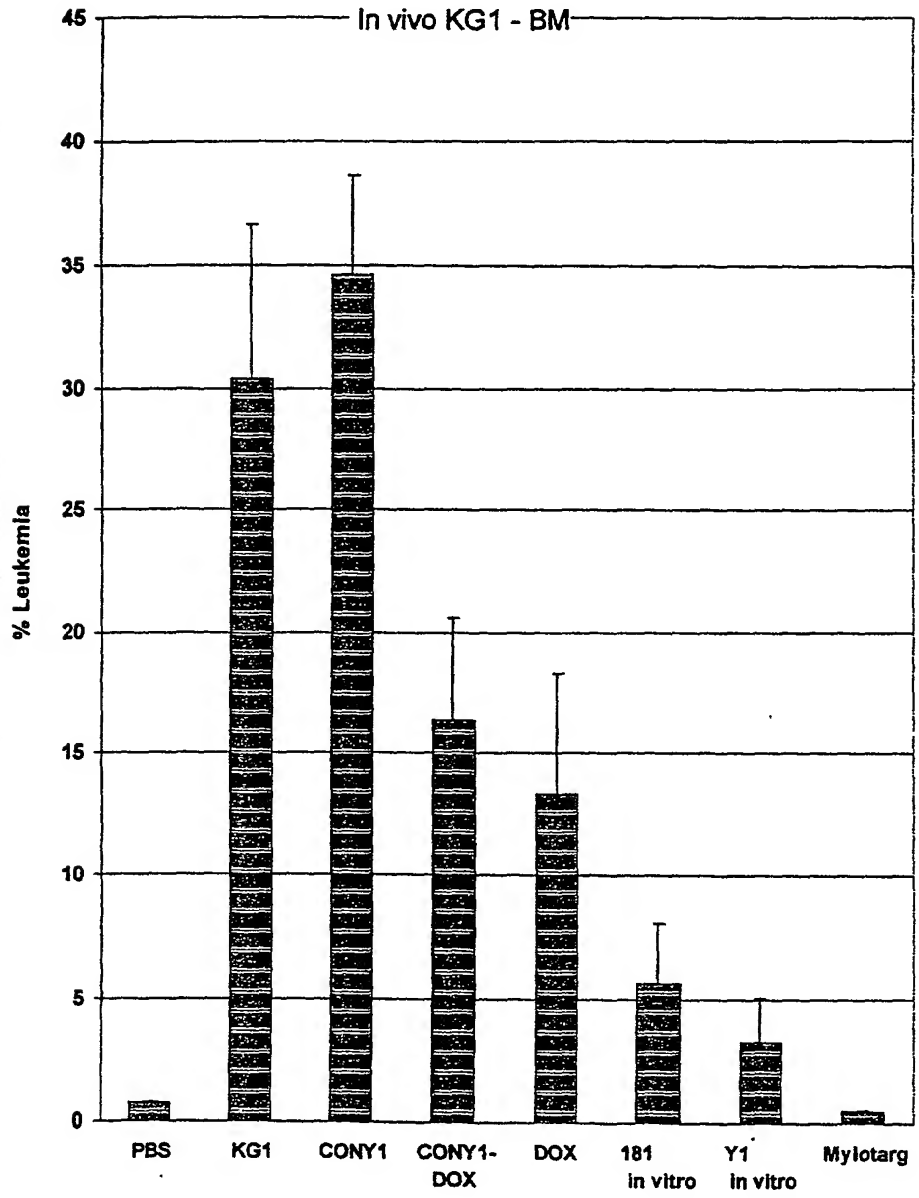
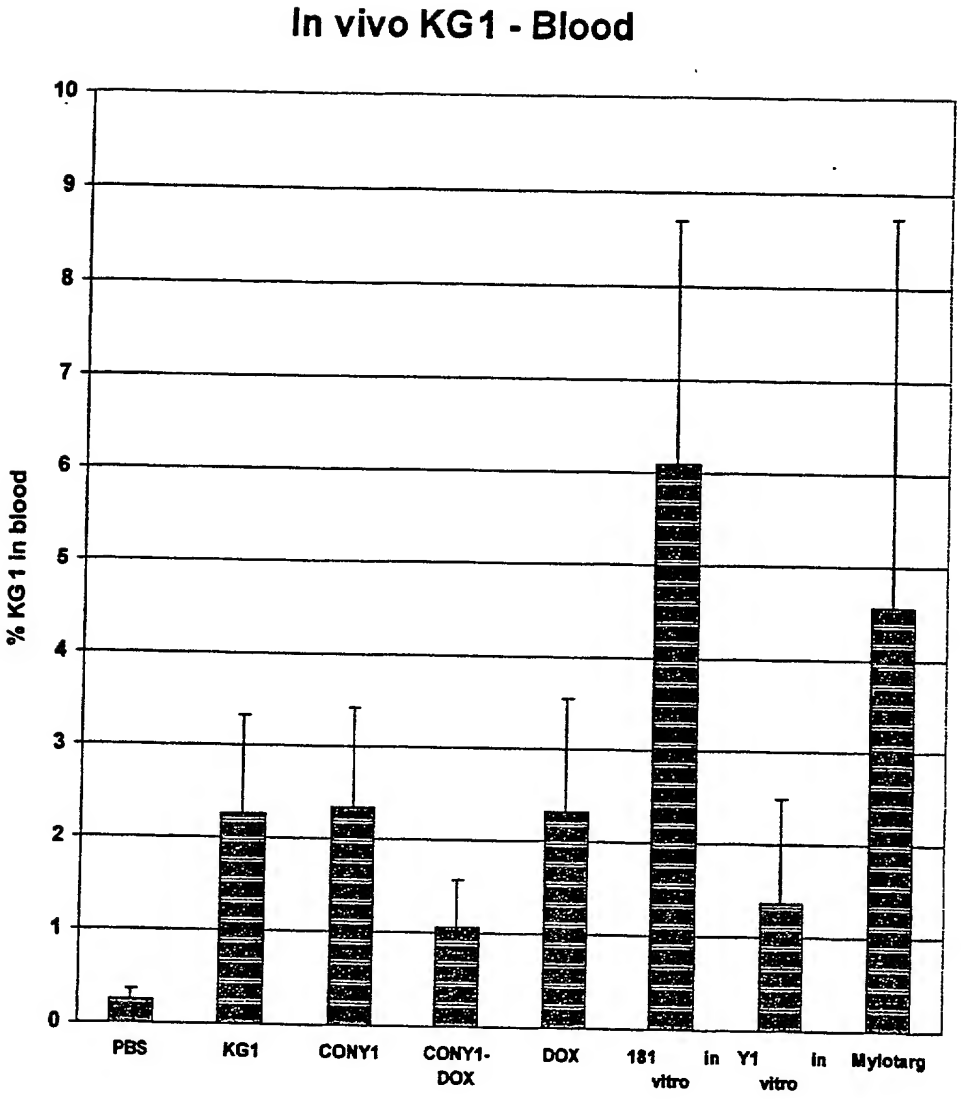


FIG. 37



****Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.

FIG. 38

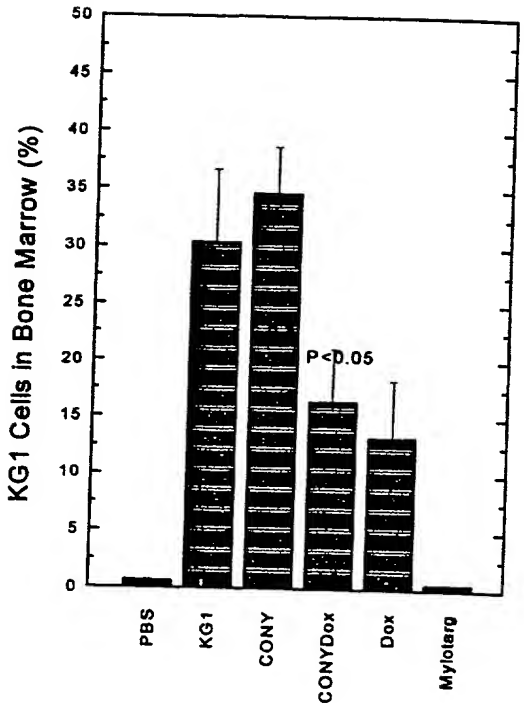

KG1 Cells in Bone Marrow (%)
50
45
40
35
30
25
20
15
10
5
0
P<0.05
PBS
KG1
CONY
CONYDox
Dox
Mylotarg

FIG. 39

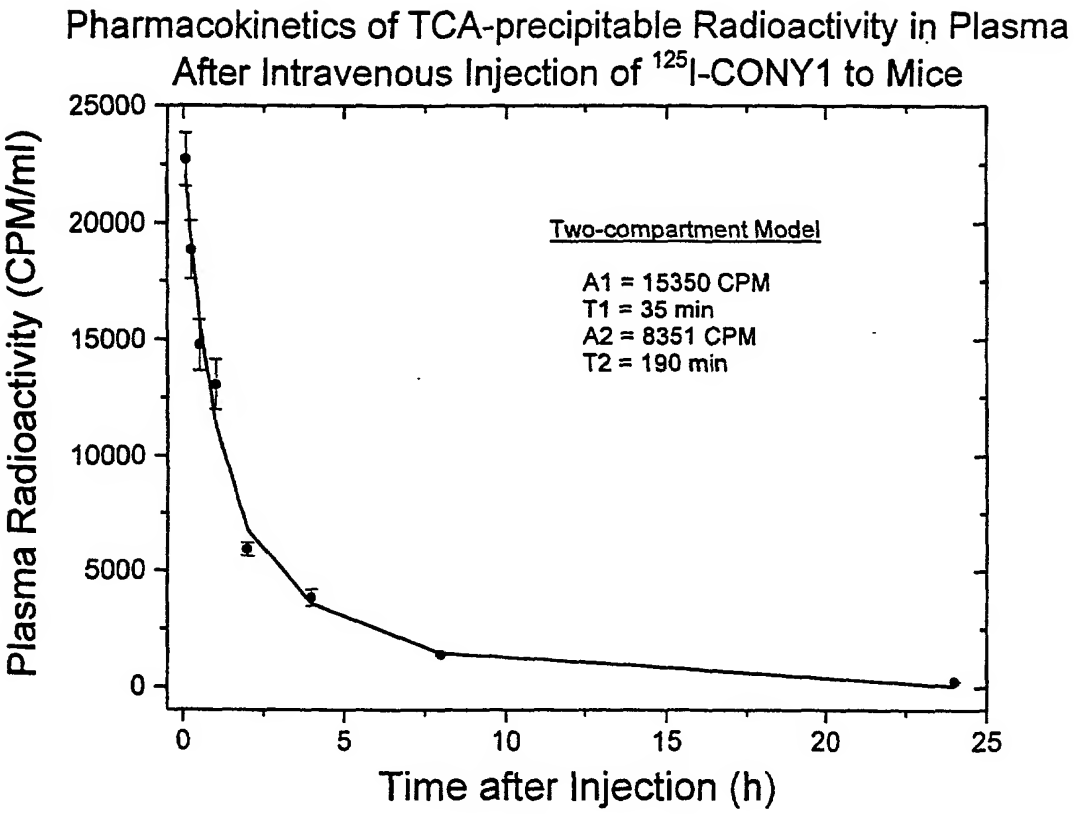

Pharmacokinetics of TCA-precipitable Radioactivity in Plasma
After Intravenous Injection of 125I-CONY1 to Mice
Plasma Radioactivity (CPM/ml)
Time after Injection (h)
Two-compartment Model
A1 = 15350 CPM
T1 = 35 min
A2 = 8351 CPM
T2 = 190 min
25000
20000
15000
10000
5000
0
0
5
10
15
20
25

FIG. 40

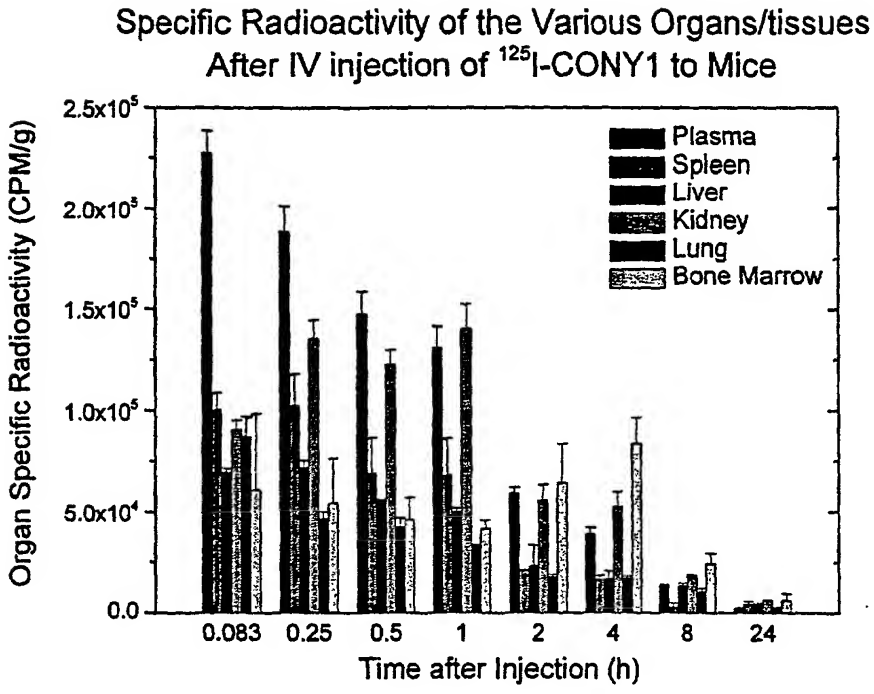

Specific Radioactivity of the Various Organs/tissues
After IV injection of 125I-CONY1 to Mice
Organ Specific Radioactivity (CPM/g)
2.5x10^5
2.0x10^5
1.5x10^5
1.0x10^5
5.0x10^4
0.0
Plasma
Spleen
Liver
Kidney
Lung
Bone Marrow
0.083
0.25
0.5
1
2
4
8
24
Time after Injection (h)

FIG. 41

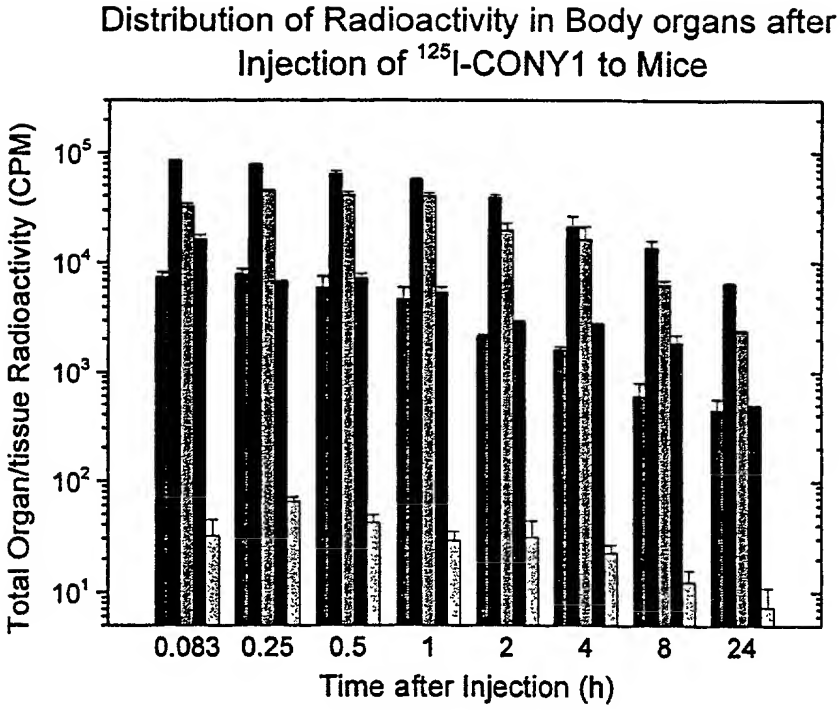

Distribution of Radioactivity in Body organs after Injection of ^{125}I-CONY1 to Mice
Total Organ/tissue Radioactivity (CPM)
10^5
10^4
10^3
10^2
10^1
0.083
0.25
0.5
1
2
4
8
24
Time after Injection (h)

FIG. 42

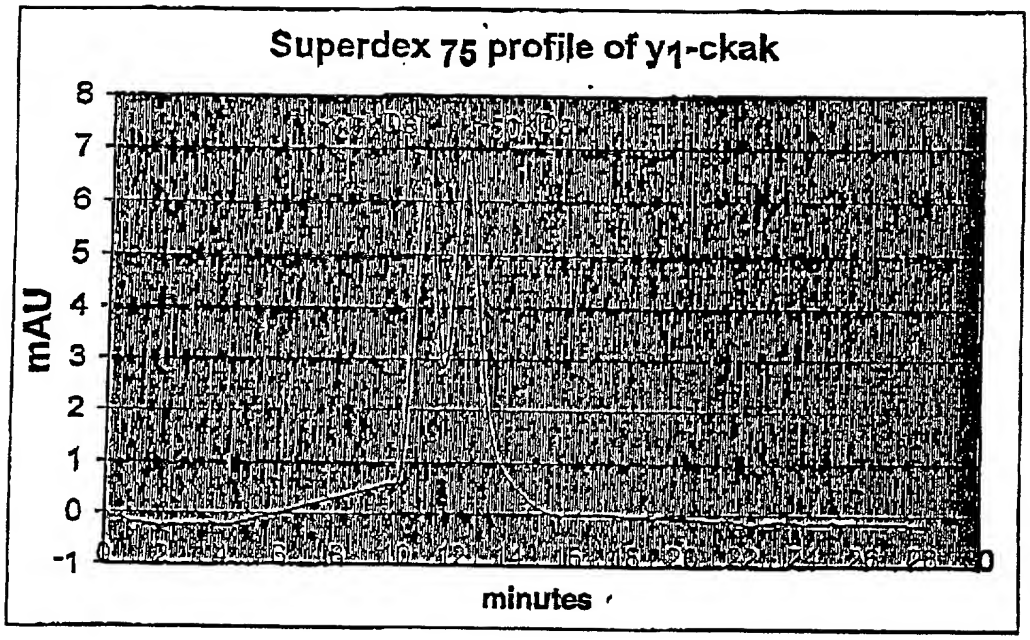


FIG. 43

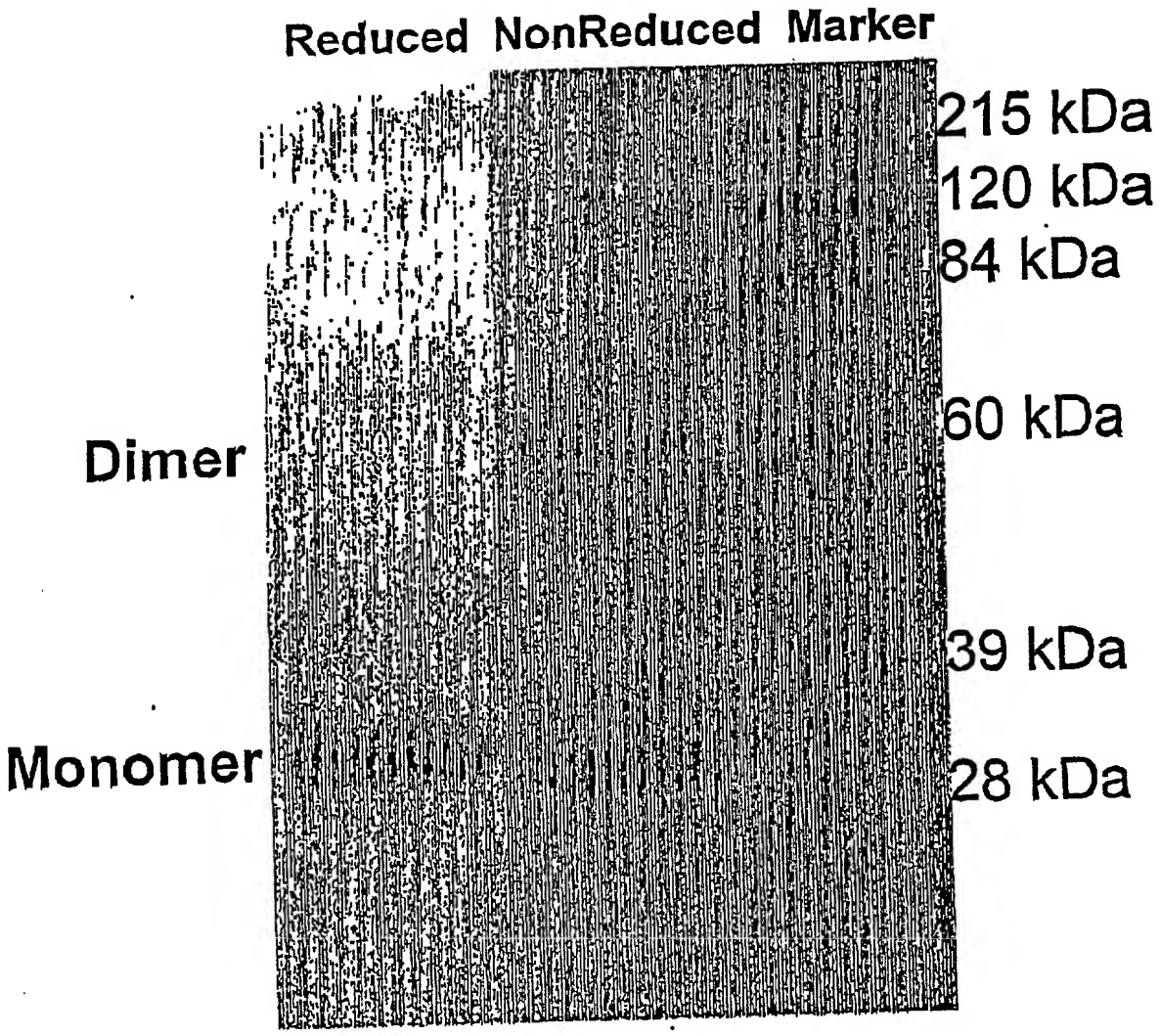
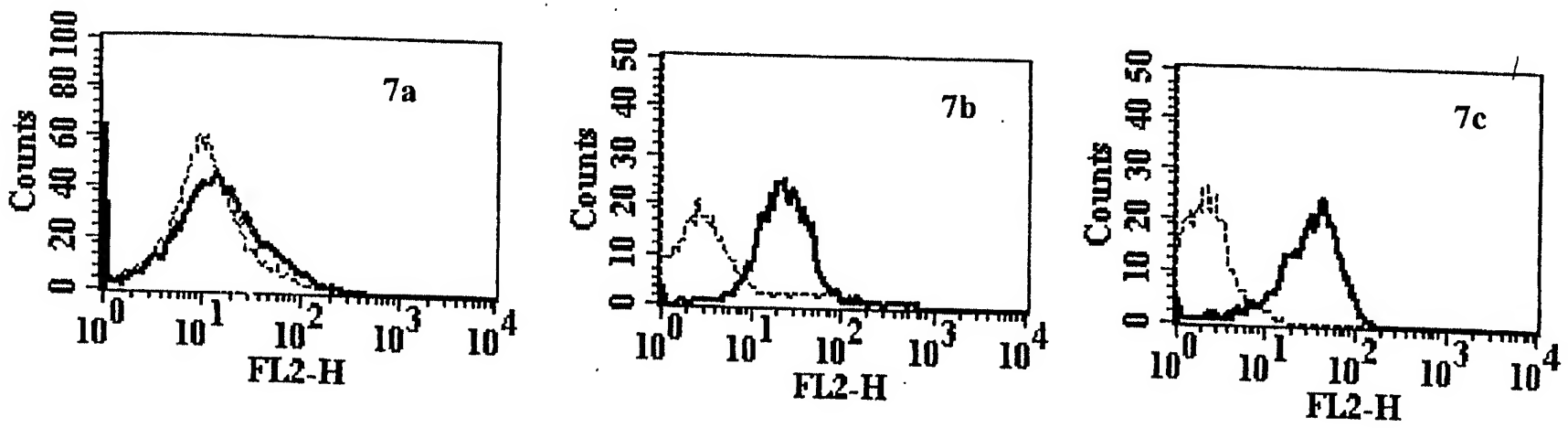

Reduced
NonReduced
Marker
215 kDa
120 kDa
84 kDa
60 kDa
39 kDa
28 kDa
Dimer
Monomer

FIG. 44



..... Control antibody

——— Y-1 antibody

FIG. 45

Epitopes of anti-GPIbα antibodies

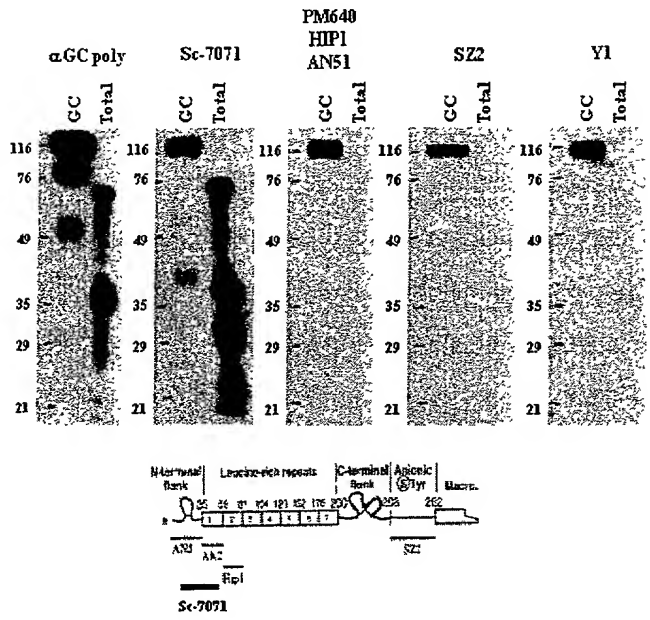


FIG. 46

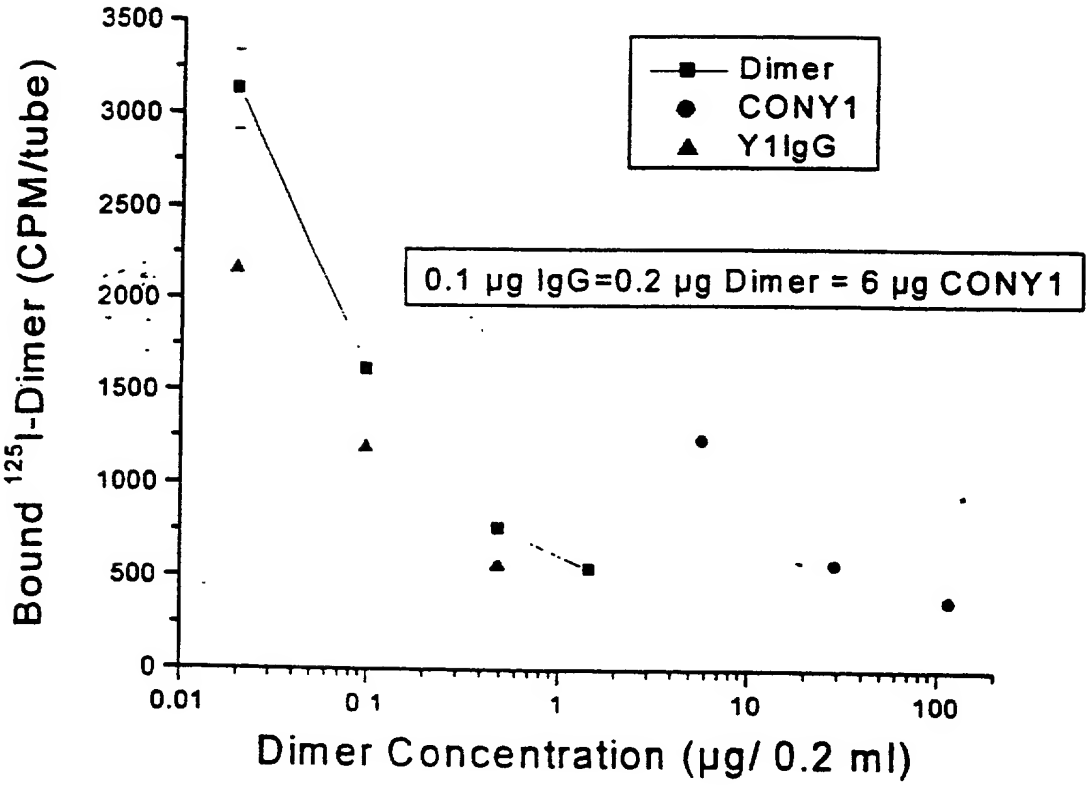

Bound ^{125}I-Dimer (CPM/tube)
3500
3000
2500
2000
1500
1000
500
0
0.01
0 1
1
10
100
Dimer Concentration (µg/ 0.2 ml)
Dimer
CONY1
Y1IgG
0.1 µg IgG=0.2 µg Dimer = 6 µg CONY1

FIG. 47

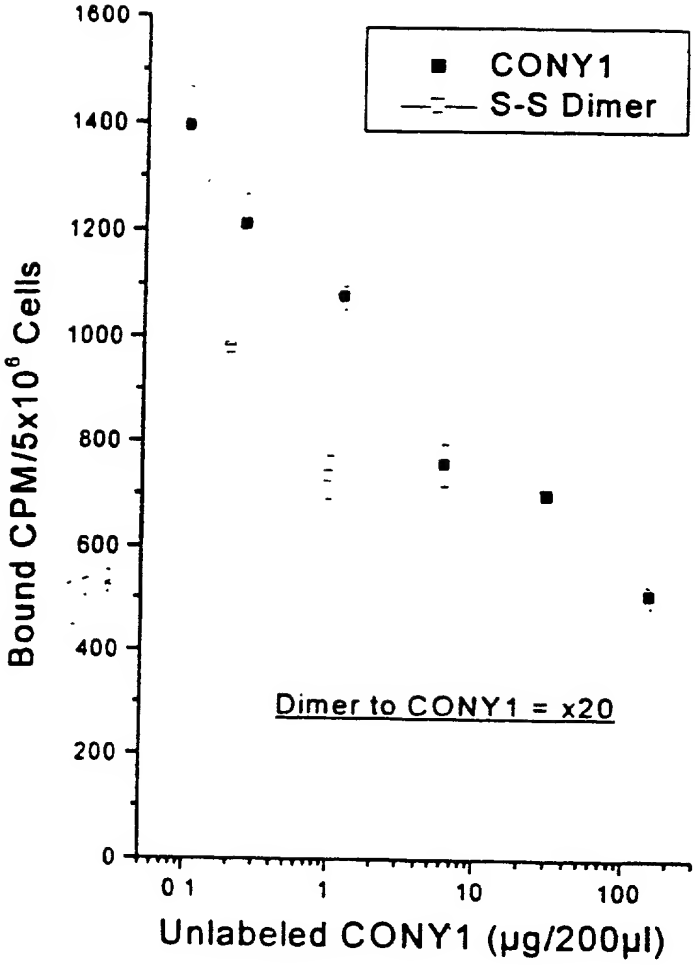

CONY1
S-S Dimer
Bound CPM/5x10^6 Cells
1600
1400
1200
1000
800
600
400
200
0
0 1
1
10
100
Unlabeled CONY1 (µg/200µl)
Dimer to CONY1 = x20

FIG. 48A: The ORF and Amino Acid Sequence of Y1-HC

SEQ ID NO: 205 (nucleic acid sequence); SEQ ID NO: 206 (amino acid sequence)

```
1       ATGGCCTGGGCTCTGCTGCTCCTCACCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1        M  A  W  A  L  L  L  L  T  L  L  T  Q  D  T  G  S  W  A  D

61      ATCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCTCC
21       I  Q  L  V  E  S  G  G  G  V  V  R  P  G  G  S  L  R  L  S

121     TGTGCAGCCTCTGGATTCACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41       C  A  A  S  G  F  T  F  D  D  Y  G  M  S  W  V  R  Q  A  P

181     GGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61       G  K  G  L  E  W  V  S  G  I  N  W  N  G  G  S  T  G  Y  A

241     GACTCTGTGAAGGGCCGATTCACCATCTCTAGAGACAACGCCAAGAACTCCCTGTATCTG
81       D  S  V  K  G  R  F  T  I  S  R  D  N  A  K  N  S  L  Y  L

301     CAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101      Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C  A  R  M  R  A

361     CCTGTGATTTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGCTTCCACCAAGGGCCCA
121      P  V  I  W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P

421     TCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141      S  V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L  G

481     TGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG
161      C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L

541     ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC
181      T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S

601     AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201      S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N

661     CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACT
221      H  K  P  S  N  T  K  V  D  K  R  V  E  P  K  S  C  D  K  T

721     CACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACTGTCAGTCTTCOTCTTC
241      H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F

781     CCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTG
261      P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V

841     GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281      V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E

901     GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301      V  H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V

961     AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
321      S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V

1021    TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC
341      S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P

1081    OGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
361      R  E  P  Q  V  Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V

1141    AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381      S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S

1201    AATGGGCAGCCGGAGAACAACTACAAGACCACGTCTCCCGTGCTGGACTCCGACGGCTCC
401      N  G  Q  P  E  N  N  Y  K  T  T  S  P  V  L  D  S  D  G  S

1261    TTCTTCCTCTATAGCAAGCTCACCGTGCACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421     F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F

1321    TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441      S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L

1381    TCTCTGGGTAAATGA
461      S  L  G  K  *
```

FIG. 48B: The ORF and Amino Acid Sequnce of Y1-LC

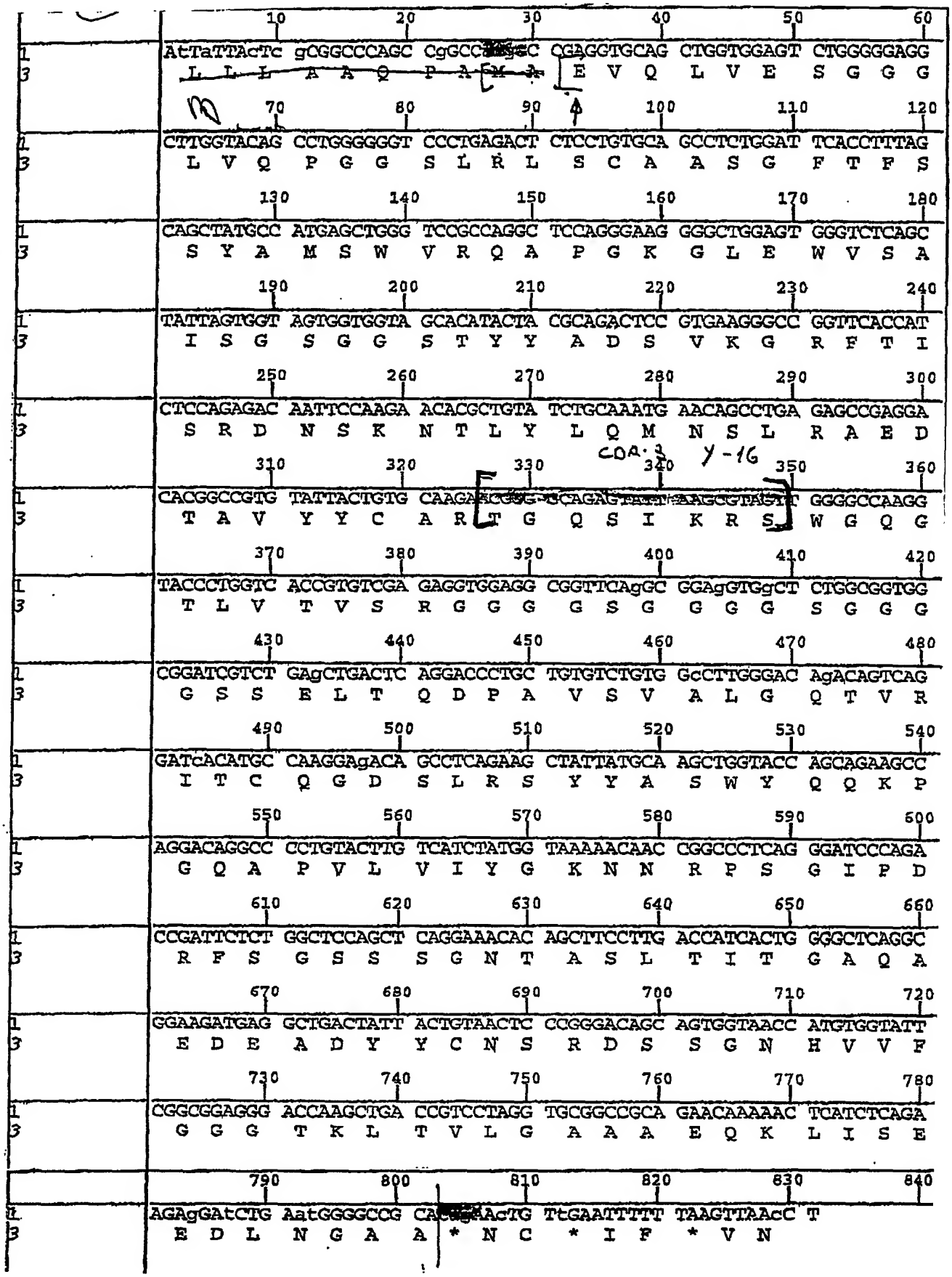
SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)

```
1       ATGGCCTGGGCTCTGCTGCTCCTCACCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1        M  A  W  A  L  L  L  L  T  L  L  T  Q  D  T  G  S  W  A  D

61      GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21       A  E  L  T  Q  D  P  A  V  S  V  A  L  G  Q  T  V  R  I  T

1212    TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41       C  Q  G  D  S  L  R  S  Y  Y  A  S  W  Y  Q  Q  K  P  G  Q

181     GCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTC
161      A  P  V  L  V  I  Y  G  K  N  N  R  P  S  G  I  P  D  R  F

241     TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81       S  G  S  S  S  G  N  T  A  S  L  T  I  T  G  A  Q  A  E  D

301     GAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGA
101      E  A  D  Y  Y  C  N  S  R  D  S  S  G  N  H  V  V  F  G  G

361     GGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121      G  T  K  L  T  V  L  G  Q  P  K  A  A  P  S  V  T  L  F  P

421     CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141      P  S  S  E  E  L  Q  A  N  K  A  T  L  V  C  L  I  S  D  F

481     TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161      Y  P  G  A  V  T  V  A  W  K  A  D  S  S  P  V  K  A  G  V

541     GAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181      E  T  T  T  P  S  K  Q  S  N  N  K  Y  A  A  S  S  Y  L  S

601     CTGACGCCTGAGCAGTGGAAGTCCCACAAAAGCTACAGCTGCCAGGTCACGCATGAAGGG
201      L  T  P  E  Q  W  K  S  H  K  S  Y  S  C  Q  V  T  H  E  G

661     AGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCATGA
221     'S  T  V  E  K  T  V  A  P  T  E  C  S  *
```

FIG. 49

```
    1          11         21         31         41         51
    |          |          |          |          |          |
  1 EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYAMSWVRQA PGKGLEWVSA ISGSGGSTYY    60
 61 ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAR[YA KTLMRQYSL]W GQGTLVTVSR   120
121 GGGGSGGGGS GGGGSSELTQ DPAVSVALGQ TVRITCQGDS LRSYYASWYQ QKPGQAPVLV   180
181 IYGKNNRPSG IPDRFSGSSS GNTASLTITG AQAEDEADYY CNSRDSSGNH VVFGGGTKLT   240
241 VLGAAAEQKL ISEEDLNGAA
```

FIG. 50



Y16 SEQ ID NO: 210

FIG. 51

Sequence of Y1-Biotag (SEQ ID NO: 211)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ

41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL

81 YLQMNSLRAE DTAVYYCARM RAPVIWGQGT LVTVSRGGGG

121 SGGGGSGGGG SSELTQDPAV SVALGQTVRI TCQGDSLRSY

161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA

201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG

241 GGLNDIFEAQ KIEWHE

FIG. 52

Y1-cys-kak scFv (SEQ ID NO. 212)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGGSGGGG SSELTQDPAV SVALGQTVRI TCQGDSLRSY
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK